PROBLEMS OF AGING

Transactions of the Fifteenth Conference
January 20, 21 and 22, 1953, Princeton, N. J.

Edited by

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NATIONAL INSTITUTES OF HEALTH, BETHESDA, AND
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MEMBERS AND GUESTS OF 1953 CONFERENCE ON PROBLEMS OF AGING

Front row Albert I Lansing, George B Wislocki, Roy G Hoskins, Anton J Carlson, F. Bourliere, E V. Cowdry, Warren Andrew *Second row* Bacon F Chow, Peggy Kubie, Janet Freed Lynch, Ephraim Short, Nathan W. Shock, Harold E Himwich, Lawrence K Frank, Earl T Engle, Eyleen A. Masser, Frank Fremont-Smith *Third row*. Henry S. Simms, George O. Gey, Robert J Havighurst, Jerome Gross, Frederick L. Hisaw.

THE JOSIAH MACY, JR. FOUNDATION CONFERENCE PROGRAM

WHEN I WAS on a destroyer out at Bikini in 1946 I was fascinated listening to our radio operator as he tested communication equipment. He would ask another ship through his radio, "How do you hear me?" and the answer often would come back, "I hear you Nine-Nine-Nine." That meant that everything was satisfactory. Of the three nines, one was for intensity, one for clarity, and one for meaning.

The Josiah Macy, Jr Foundation has organized and devoted a large portion of its resources to the support of its Conference Program because the officers are cognizant of the fact that there is considerable obstruction to communication and mutual understanding across the disciplines and specialties, and that this, in fact, is one of the major factors delaying scientific advance. We feel that there are psychological, as well as semantic factors contributing to the difficulty of communication, people, even in arguments with one another, are too much inclined to make statements *at*, rather than to communicate *with*, others I think that we are inclined to forget, though, that the real question is, are these words and statements those which are likely to convey to the listener the whole or even a small part of what I would like to express

I have a feeling that we should be very much concerned with the other fellow's receiving set and not only with our own transmitter If the other person doesn't seem to understand us, it may not be enough merely to increase the power of our transmission; we must try to find the obstruction in his receiving set, and see what kind of filters and resistors he uses. So, if we call out to the interprofessional No-Man's-Land, "How do you hear me?" and the reply comes back, "I hear you Nine-Nine-Nine," we have the beginning of communication What we try to do in these conferences conducted by the Foundation is to set the stage for meaningful communication

With the [illegible]
and with [illegible]
comes e[illegible] isolation of the several branches of science from one another is a serious obstacle to scientific progress. Nowhere in science is the need for "combined operations" more evident than in medicine Today, to be effective, medical research and practice must embrace data from all the disciplines including nuclear physics at one end of the spectrum and cultural anthropology at the other, for

advances in one field are frequently dependent upon knowledge derived from quite another discipline.

Although the fertility of the multidiscipline approach is thus recognized, universities, and scientific societies and journals which are usually restricted to one small area of a field in their coverage, have not yet made adequate provision for channels of interdisciplinary communication. We do not wish to compete with the formal scientific meetings or with the scientific journals which have established patterns and formats for the presentation of material. Our purpose at the meetings is to keep an informal atmosphere and to encourage the exchange of methods, research plans, concepts and difficulties, which cannot be done if there is formal speech making.

The Foundation has endeavored to meet the need for interdisciplinary communication by bringing together for a series of two-and-a-half day annual conferences a small group of investigators, representing insofar as possible all the branches of science related to a chosen problem. Participants in these informal conferences over a five-year period develop a feeling of friendship, trust and mutual respect which in turn promotes communication, cross-fertilization of ideas and cooperation. The success of such an endeavor, however, is dependent upon full participation of all members in the discussion. Accordingly attendance at any conference is limited to twenty-five

Under the guidance of Dr Willard C Rappleye, President of the Foundation since 1942, the Conference Program has been gradually expanded and enlarged until during 1953 it included twelve different groups which meet annually to discuss a wide variety of problems in the field of medicine and the closely related disciplines Our plan is to discontinue the meetings of each group at the end of five years.

In order to share with a wider group of investigators and students the essential quality of these conferences and to give others an insight into the functions of the scientific mind, the informal nature and tempo of the discussions, as far as possible, are preserved in the published transactions

FRANK FREMONT-SMITH, M D.
Medical Director

INTRODUCTORY REMARKS

ROY G. HOSKINS
Chairman

THE WORK of this conference of the Macy Foundation has, I suppose, as its ultimate motivation, the improvement of the state of the elderly human being The problem of aging, of course, presents a number of facets, more or less distinct, often greatly overlapping. These facets have been approached in a variety of ways, sometimes in a rather hit-or-miss fashion, sometimes more systematically. I like to think of man as an integrated organism that is susceptible to study at a considerable number of emergent levels He can be thought of as a psychological organism

Then, one's interest is focused on the intrapersonal characteristics, especially the ancestral drives, the indulgences and frustrations A great deal of human welfare hinges on those issues.

He can be thought of at the social level, in his relationships to the environment, and especially the human environment. His interpersonal relationships come into that.

We have dealt with man at the social level, considering his relationship to other people and to society That involves a good many economic issues of one sort or another. We have dealt with those somewhat systematically, at a previous conference We have also covered problems of nutrition and consumption

There is one aspect that is rather notable for its omission, and that is man and his cosmic environment, his cosmologies and theologies. By common consent, we have stayed off that ground. It has never been quite clear in my own mind why we have, but we have.

We have dealt with the organ systems which, at the physical level, make up the individual The kidneys and skin are two of the organs that have had somewhat special attention, depending, again, I think, especially on the personality of this group In the years during which Dr MacNider was the moving spirit, we heard a great deal about the kidney, because that was the field in which he had worked Dr Cowdry has often directed attention to the skin

At the present conference, we have logically come down pretty well to the foundation level that is to say, the anatomy and physiology of the cell I think it *might* be desirable if we stick fairly closely to the cellular level, both as to structure and function Perhaps it is wise

that we do come to this level of consideration at this time, because in the past decade, as, of course, you all know, there has been very much advancement in instrumentation. We have electronmicroscopy, which is developing, the finer cytologies, tissue cultures, and things of that sort. We know a great deal more about enzyme chemistry. Then there have been important developments in comparative biology, you might say, things we may learn from other organisms, rather than man himself

In of the thought we may have, I think we would be wise to stay pretty ell by the old pattern that has gone through these discussions throughout all fifteen years, the aim being not so much to determine what is known, as what is not known, and how we can most wisely proceed to fill the gaps in our knowledge.

That would logically bring up some discussion of the newer techniques and what these techniques can teach us. The potentialities and weaknesses of these techniques would be other problems that would logically come in at this time.

Before proceeding with our discussion it may be well to become briefly autobiographic, and introduce ourselves. Nathan, will you start the ball rolling?

NATHAN W. SHOCK: I am Chief of the Section on Gerontology of the National Heart Institute, National Institutes of Health. Our research laboratories are operated in collaboration with the Baltimore City Hospitals in Baltimore. After receiving my M.S in organic chemistry at Purdue University, I completed my graduate training at the University of Chicago under Dr. A J. Carlson, Dr. A B. Hastings and Dr. L. L. Thurstone. My major research interest was the evaluation of physiological and biochemical factors influencing behavior. After completing my graduate work, I spent ten years at the University of California with Professor Harold E. Jones working on the physiological aspects of adolescence and teaching physiology in the University of California Medical School. In 1941 I accepted an appointment in the Unit on Gerontology which had just been established in the National Institutes of Health by Dr. W. Henry Sebrell I came to the position with no real knowledge of aging, but thanks to Dr. Cowdry's excellent book and the conferences on aging, sponsored by the Macy Foundation as well as the continued support of the Public Health Service, I found the field most stimulating and of widening interest.

EPHRAIM SHORR: It was just about three months ago at another Macy Conference that I had to provide reasons for having become interested in circulatory homeostasis; and now if I provide equally good and sufficient reasons for my interest in gerontology, I hope it will not appear that I am fickle in my attachments

Specifically, my interest in this field may be said to stem from my relationship to Frank Fremont-Smith and the Josiah Macy, Jr Foundation About twenty years ago, when I was asked to set up an Endocrine Clinic at The New York Hospital, I began with Dr. Papanicolaou to use vaginal smears as objective indices in the study of disturbances in menstrual function in women. A few years later, discussions with Dr Fremont-Smith of the problems involved in the meno-

the aging of the blood vessels in relation to hypertension At about that time, I was invited to join this group. The stimulation that has come from these meetings, the investigational assignments which stemmed from them and the associations formed here have all been factors entering into my orientation towards gerontology.

E. VINCENT COWDRY: I came into the study of aging by way of arteriosclerosis About 1932 Dr Ludwig Kast came to Washington and asked the Division of Medical Science of the National Research Council about the advisability of preparing a comprehensive statement concerning arteriosclerosis He was a pioneer, I think, in realizing that the time for the concentration of attention on chronic disease had arrived, that we must work on a long-term basis, and that the preventive medicine of chronic disease should be our goal The acute diseases had been tackled pretty successfully, while the chronic diseases had been neglected That was his first point of view.

His second point was that this statement must not be narrow, but that it must represent all aspects of the

It was the first international study, as far as I know, of arteriosclerosis

I should like to take a few moments to trace the sequence of developments, if I may This work on arteriosclerosis showed that it was desirable to broaden the basis of our in-

that we do come to this level of consideration at this time, because in the past decade, as, of course, you all know, there has been very much advancement in instrumentation. We have electronmicroscopy, which is developing, the finer cytologies, tissue cultures, and things of that sort. We know a great deal more about enzyme chemistry. Then there have been important developments in comparative biology, you might say, things we may learn from other organisms, rather than man himself. The genetic aspects, again, come into the picture.

In proceeding from this point to the development of the thought we may have, I think we would be wise to stay pretty well by the old pattern that has gone through these discussions throughout all fifteen years, the aim being not so much to determine what is known, as what is not known, and how we can most wisely proceed to fill the gaps in our knowledge.

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that the Social Science Research Council had set up a committee on social adjustment in 1945, I talked with Professor Burgess, who was chairman of that committee, and we agreed that we would try to establish a subcommittee on social adjustment in old age. He and I together developed a program for a committee on social adjustment in old age. That marked my first attempt to go into this field

I think I am the only social scientist in the group here, and for the purpose of this conference, I believe I ought to remember that I took my degree in chemistry. I shall try to be a chemist for the next couple of days

ALBERT I LANSING: I am a member of the staff of the Washington University School of Medicine. I received my training in zoology, have done most of my work in cell physiology, and make my living as an anatomist But throughout these three fields, my activities in problems of aging have carried through as a thread As a matter of fact, my interest in gerontology goes back to quite a long time ago. As a junior in high school, I was very much impressed [illegible] the concept of intestinal pu[illegible] on I did experiments on m[illegible] *ilus* as a means of altering [illegible] whether they will delay my demise or not

But, speaking of gerontology, I think I should point out that I have a very soft spot in my heart for both Dr. Frank Fremont-Smith and the Macy Foundation I don't know whether all of you are aware of the fact that the interest of Dr. Fremont-Smith and the support of the Macy Foundation made possible in 1941 the beginning of my career as an active gerontologist with Dr Cowdry. It was directly implemented through that support

FREDERICK L HISAW. I think probably for this discussion my remarks ought to be limited to things related to the aging process

By training and thought, I am a zoologist and am particularly interested in the physiology of reproduction When I was invited to take part in the discussions of this group a number of years ago, I had several rather long talks with Dr. MacNider concerning what contributions if any I might be able to make to the group

A number of things were mentioned at that time One of these, of course, was the fact that the physiology of reproduction has a very definite relationship to aging: coming into

vestigations by including a study of aging along the same lines. That was done, and that also was an international study.

The arteriosclerosis study culminated in 1933, and the first edition of the book on aging appeared in 1938.

BACON F. CHOW: I am a biochemist and am a novice in this field. I am here to learn about gerontology I had the courage to accept the invitation to attend this conference because I was told that one of a participant's prerogatives is to speak on a subject on which he has some ideas but on which he hasn't worked.

For a number of years I have been interested in the biochemistry of growth. Well, we know an animal grows when he is put on a good diet But what do we mean biochemically when we say animals grow? They increase in body weight, they increase in length, and they increase in a number of other things. We tried to go into a little more detail by studying the changes in the body composition and, in particular, some of the enzymes and proteins. Later on we studied the biochemical changes due to stress. When we looked the data over, there seemed to be a common denominator as far as the biochemistry of growth and stress was concerned. And in thinking it over, growth is one phase of life, whereas aging is another phase of life.

Isn't it, therefore, conceivable that some of the knowledge we have gained about the enzymes and the composition of the body could be applied to the process of aging?

ROBERT J. HAVIGHURST: After completing graduate work in the fields of chemistry and physics, I became interested in education, and taught in experimental educational programs at the college and secondary school levels for several years Then I joined the staff of the General Education Board (Rockefeller Foundation) and soon became interested in child and adolescent development, largely under the stimulation and guidance of Lawrence Frank. When, in 1941, I went to the University of Chicago, I knew then that I wanted to be interested in human behavior and human development throughout the life cycle At Chicago my research has been in the area of social psychology of children, adolescents and adults. As a member of the Committee on Human Development at the University of Chicago, I work on an interdisciplinary team with people from the departments of psychology, anthropology, sociology, physiology, and education. When I learned

Cowdry's proposal came to us. I became entranced with the problem because we couldn't define it. What do we mean by aging? Also I became entranced by Bill MacNider's observations on the cells of the liver and kidney tubule after severe poisoning, when the animal just barely survived and came out with flattened syncytial cells in these tissues, which were then resistant to a great variety of poisons. He pointed out that he had seen these same flattened syncytial cells in liver and kidney tubule, appearing spontaneously only in the very aged dog. I wondered: "Do these liver and kidney tubule cells in the old animal and in the animal recovering from this injury, still perform their functions even though they are changed as tubule cells and as liver cells?" They had acquired a resistance, but at what loss? There must be some loss. I have never seen an answer to that. It seemed to me that since those cells appeared spontaneously in the aged animal, there was a problem there.

ROY G. HOSKINS: In my present job I am engaged in looking after medical and biological contracts in the New England area for the Office of Naval Research.

I was working in endocrinology at the time I was invited into this group, and also was engaged in research on schizophrenia at the Worcester State Hospital. It was perhaps because of my endocrine interest that I was invited to come aboard. However, being with the group and watching its development has been a very delightful experience. Even at that time the subject of [illegible] knowr [illegible] impres [illegible] in the [illegible], has been very effective, because the wilderness has become rather densely populated now with groups who are giving special attention to this special subject of aging.

WARREN ANDREW: I am professor of anatomy at Bowman Gray School of Medicine. At a fairly early age, I became interested in reading in the field of studies on aging, and, like Dr. Lansing, I read Metchnikoff, *The Prolongation of Life*, but I didn't try to carry out any practical experiments as a result of that. I was content with the theory. And I read *Age, Growth and Death*, by Robert Sedgwick Minot. I can't recall the author, but somewhere I found a work called *Natural Salvation*, which was written by a physician who felt that a very great deal

sexual maturity, functional reproductive life, and then weakening of reproductive processes, had a direct bearing on questions of aging. And then, more particularly, the point I presented, which he thought was most worth while, was my interest in the aging of certain tissues. There are certain tissues, such as the corpus luteum of the ovary, the placenta, and other tissues associated with the reproductive process, which come into existence at each estrous cycle or pregnancy, and which have a very definite functional history, including changes associated with age and finally senility and involution and disappearance.

So, I have been quite active for the last fifteen years in an attempt to study what it is that brings a tissue into its functional existence, what it is that maintains the physiological *process, and why it is that the physiological action of a particular* structure finally ceases. To me it seems quite probable that, if one could get an idea as to the nature of the aging process in a particular tissue, it might contribute to a better understanding of the aging process in general

So, if I have made a contribution to this group, it has been along those lines

JEROME GROSS: I am associate biologist in Dr. Walter Bauer's Arthritis Study Group at the Massachusetts General Hospital and am also research associate on the staffs of the Department of Biology, MIT, and the Department of Medicine at Harvard Medical School.

My interest, primarily, is in the molecular and colloidal organization of tissues and their reactivity, with particular reference to connective tissue I became interested in this field in medical school, maintaining an interest in rheumatic disease and in the application of physical and chemical principles and methodology to biological problems. My interest has not been primarily gerontological However, our studies on the development and alteration of connective tissue and fibrogenesis, and of collagen have some bearing on the widespread mesenchymal changes in aging processes and degenerative diseases

FRANK FREMONT-SMITH: I am medical director of the Josiah Macy, Jr. Foundation. I was trained in neurology and neurophysiology, got a little chemistry on the side, and then moved over toward psychiatry. That was my general background I didn't know anything about aging and had never thought about it until

I occasionally dip back into less formal literature on aging, and must admit that about a year ago I read a rather fanciful book called *Old Age: Its Cause and Prevention*, which was written by a San Francisco author in the early part of this century, the main gist of which was that he had discovered a system of exercises in bed which were guaranteed to prevent or defer for a very long time the onset of aging. He had a before-and-after picture of himself He looked like a rather senile and run-down character at 51 years of age, and like a very spry and agile person at 75.

So the fundamentally scientific aspects of aging have been fascinating to me. One aspect of studies of aging that has appealed to me all along is that the field and the various ramifications of it have no apparent end, and it is a field in which a great many divergent types of persons and divergent types of scientific effort can come together

F. BOURLIÈRE: I am associate professor of medical biology in the Faculty of Medicine of the University of Paris A naturalist by avocation, I got my M D degree in the University of Paris in 1940 and afterwards graduated in physiology, biochemistry and animal biology in the Faculty of Science of the same university During my internship in the Necker Hospital in Paris, I became interested in gerontology when working with Professor Binet, the present dean of our medical school, whose interest in old age problems is very great He encouraged me to devote most of my time to gerontological research, and the establishment of a new department of medical biology in our medical school gave me the opportunity to start research of my own on the comparative physiology of growth and aging

My present work is mainly concerned with the comparative study of growth and aging patterns in cold-blooded vertebrates, as compared with warm-blooded vertebrates, and with the study of those mammals whose poor temperature regulation is associated with a very long life-span Our approach is both ecological and physiological and I hope it will some day become biochemical

ANTON J. CARLSON: I have worked in physiology as a member of the faculty of the University of Chicago for 48 years. Of course I realized very early, long before most of you were born, that the basic problems in biology and in disease all bear on the unsolved problems of aging — all of them, including basic

could be done to prolong a healthy life for a period of hundreds of years at some time in the distant future. I resolved then to keep an interest in this subject and carry out work in studies on aging.

When I went to Brown University to do graduate work, a good many people were sitting about at microtomes having a great deal of trouble sectioning material and putting it on slides, and I decided definitely that I would not go into histological or cytological work, so I began to work on the problem of the senescence of Cladocera, and studied the waning of their reproductive capacity as evidenced by the increasingly smaller number of babies that could be found in the jars of these animals from one day to the next. I began to notice some morphological changes with age. But the very next year I had started in histological work, because it was an intriguing thing. I had to do my own technical work, of course, and was using the microtome. This gave rise to a Ph D problem in zoology.

After I had finished my graduate work and had begun teaching in anatomy, I received a great impetus in finding that there was a group that was really interested in gerontology and that *Problems of Aging* had been published. This gave me an impetus because at that time I had somewhat of a feeling that studies on aging were not entirely "respectable" in scientific work. When I saw that such a fine group had banded together to publish this book and that many people were definitely interested, and in a fundamentally scientific way, I felt it was quite in place to go ahead.

Then, in 1941, the Wistar Institute of Anatomy started on a project. It was rather interesting in showing the need for studying aging because Dr Farris wanted to study the effects of emotional crisis in the lives of rats, a crisis brought about by a terrific noise directed at the rats He wanted to see what would happen to the animals throughout their life histories as a result of this type of crisis But he found that he had no control material because nobody knew exactly what happened to the tissues of rats as the rats grew older normally, so he asked about twenty to twenty-five people to collaborate in studying the changes in the normal tissues with age That greatly appealed to me, and proved to be a very helpful thing for my own work. With the possibility of having graduate students, which came up about four years ago, I felt that it was possible to expand a little and get into some of the cytological problems that had been appealing

was also one of his early water-balance guinea pigs Following my early contacts with these men, I landed at The Johns Hopkins Medical School and was invited to work with Dr. Joseph C Bloodgood on tumor pathology and on the cultivation of tumors (1) in the laboratory of Dr. Warren Lewis, of the Department of Embryology of the Carnegie Institution of Washington in Baltimore To my knowledge, this was his first sojourn in tumor cytology. Dr. Lewis was one of the leading cytologists of the day. I have received much stimulation from him since that time.

One of the problems at that time was this business of isolating in culture cell strains so that one could study these target end organs, so to speak, outside the body. For years using such biological material (i e, cultured cell strains), Mrs Gey and I have been faced with the problem of serving the interests of many. For a while it seemed as though one of my chief functions in life was to direct a laboratory with cultured cell strains to help satisfy the scientific curiosity of others besides myself (2).

After a year at Hopkins, working with Dr Lewis, I moved to the Columbia Hospital in Milwaukee, Wisconsin, with the encouragement of a host of Hopkins people who were there; especially Dr George L Streeter, Dr. Joseph C Bloodgood, and Dr. Warren H Lewis These men encouraged me to work with one of the Hopkins men, Dr. John L. Yates, then in Wisconsin I spent a sojourn of some six years in Wisconsin where I set up a cancer research laboratory at the Columbia Hospital in Milwaukee Through correspondence and direct contact, I got much guidance from Dr Ludwig Hektoen, Dr Preston Kyes, Dr. R R. Bensley, and Dr A. Maximow This encouragement from the group at Hopkins and of local men in Wisconsin, especially Dr Banting and Dr Thalhimer, and contacts with Dr Alexis Carrel and Dr James Murphy of the Rockefeller Institute, enabled me to continue, even in a period when the kind of things that we were doing were quite unpopular, at least in some quarters

Since that time, I have continued to use primary explants of tissues and isolated cell strains maintained in continuous cultures as our chief objects of study These cells can readily be chosen from young and old hosts, and it is, of course, a natural thing to ask whether these isolated cells are fundamentally different from one another Because my support came chiefly from organizations interested in cancer biology,

problems in sociology. Back in 1912 or 1913 I carried out a series of experiments on the human stomach. Extending that work, I found in checking the hunger contractions of the empty stomach of adults against the hunger contractions of premature human infants, that the period of rest of the empty stomach in the infant was only about five or ten minutes, and that in most of the adults it was from two to three hours, and *the older the individual, the longer the rest period between* the hunger contractions. That was not only true of man, but also of the dog. That is a phenomenon of functional aging, and so is the decrease in gastric secretion.

One of the last pieces of work of interest to me was to challenge some experiments by one of our members, Dr. McCay. He had controlled rats, litter mates, and he fed some of them so little that they grew very slowly, but their life span was much longer than that of their brothers and sisters who had received as much food as they wanted I visited my friend, Dr. McCay, at Cornell University and asked him, "Do you know whether you have really lengthened the life of these undernourished rats or whether the life of the rats who just ate and sat, without any struggle for food, was shortened?" So we did experiments on periodic fasting, or putting so much indigestible roughage into the food that the rats couldn't overeat, and that lengthened their life.

I have profited by association with this group. This time, however, I came here, Dr. Fremont-Smith, essentially as a guinea pig, because I think I am the oldest in the group. One of you—I think it was Cowdry—says that old people are bored. If we could maintain our primitive curiosity, we would not be bored We would enjoy learning new and important things as long as our cerebrum works. And that is one of the big problems, I think, in adding years to life in a group like this, in the Gerontological Society, and in the International Congresses of Gerontology.

GEORGE O. GEY: I am at The Johns Hopkins University I began my scientific career over thirty years ago at the invitation of two people, first with Dr. Clarence C Little, of Harvard University and director of a program of experimental cancer research at Cold Spring Harbor, where I was one of his assistants in 1921, then with Dr. Edward Adolph, who had just come from Oxford. At that time, I was a graduate student at the University of Pittsburgh, where Dr Adolph was teaching zoology and physiology, and I was one of the instructors. I

There are many situations in which, after regenerative repair, the whole tissue responds to an influence which we can poorly define This influence, which we see in the host, is manifested in the form of maintenance of differentiation and a settling down It is something which one can manipulate in a test tube of cultured tissues. To get at this, one must, of course, fractionate the humors of the organism in order to understand what goes on in the host. One cannot do much of this sort of thing very easily in the host, but one can do it easily, we feel, in the test tube.

We have been very fortunate in having some altered cell situations arise in our culture work, and I should like to say briefly what they are and why we should pursue some of these lines of approach We have seen the production of malignant cells in what were previously permanent and stable lines of normal cells We are not able to account for these transformations into malignancy Nor are we able to say that this is the only way in which a cell can develop when it deviates from its normal state Someone here spoke of the flattened cells that occur in the kidney and the liver and how different they are from what they were before. Well, I can assure you that after a radiation exposure, cells are different from what they were before, and it is understandable why that should be the case. The mutagenic effects of injurious agents are well recognized

In other work that we are interested in, we have seen the loss of transplantability of a tumor cell strain, or, one might say boldly, the loss of malignancy, in cells that once were malignant This is a process that we do not understand at all

If one were to think of our laboratory as a bacteriological laboratory, one might readily understand some of these things if [illegible] of [illegible], so[illegible] [illegible] duced, some rather transitory, others ending in permanent mutations.

In all of our work, it has been necessary to resort to improved methodology, and we have tried to contribute in this direction More recently, we have developed a new method for growing cells in suspension, so that those who are interested in direct chemical analysis or other studies of large masses of cells, can carry out some of these procedures

HAROLD E. HIMWICH. I am a physician and a physiologist and so, like

I quite naturally kept my main ideas focused in that direction

However, my connection with gerontology, in the immediate past, came from an invitation from Dr. Korenchevsky, of Oxford, and from Dr. Cowdry to participate in the Second International Gerontological Congress held in St Louis in 1951. I must say I got a lot out of that meeting. I was faced at that congress, as most of you were, with more problem identification, and I am pleased to say, this no longer constitutes a big issue in my mind. At an earlier period we had too little of it in our laboratory Improved methodology plus a long experience has made problem identification easier. As a result, we have found our kind of activities more popular.

We have tried to identify specific facets of interest so that we can work along definite lines, often backing individuals to pursue them rather than less well-defined projects. This seems to me the most economical way to do it, to get a good man who has a well-defined problem, and let him run it down to a satisfactory conclusion Often-times lack of continuing support interrupts such research.

We have had many young scientists from all parts of the world in our laboratory For example, one of this group has worked directly on problems related to aging. He is Dr. Andrè Glinos, who has been studying the problem of the relationship of regeneration and neoplasia and has done some good work on the subject. I have always been interested in the relationship of aging to cancer and, therefore, sponsored this work with much interest My personal belief is that there is no direct relationship of cancer to aging

Malignant transformation can occur at a very early age in development. From the blastocyst stage tumors can arise from the extra-embryonic trophoblast resulting in the production of chorionepithelioma. From here on tumors arise from the tissues at all ages, but induction of them may require many years as compared to the briefer periods at the very beginning of life

At the present time, Dr Bang and I are much fascinated with our work on viruses As you know, viruses are organisms which require cells for their existence, and I believe that viruses have a lot to do with some of the processes of aging. Now, there is another facet of our work that I am greatly interested in, and this is the thing we call dormancy in tissues and in the organism as a whole Dr Glinos is much interested in this, also.

HENRY S. SIMMS: I am at the College of Physicians and Surgeons of Columbia University, New York City. My training was in biochemistry but I am located in the Department of Pathology.

My interest in gerontology began 25 years ago here in the town of Princeton. At that time I was working at the branch of the Rockefeller Institute that was located on the outskirts of Princeton, and I was doing physiochemical work on proteins and amino acids, but I felt that I would much rather change my field of research and work on more important problems. It seemed to me

problems I have been doing so for the last 25 years, but haven't solved either of them yet.

I have been thinking, during the conversation here today, about the difference in the situation 25 years ago relative to the problem of aging as compared with the attitude toward gerontology at the present time. Twenty-five years ago, as Dr Andrew pointed out, working on aging was not considered respectable. There was practically no scientific interest in aging. It was practically impossible to get financial support, because the foundations at that time were handicapped by shortage of funds and were forced to spread their money out for research on problems that could be worked out in a year or two. There was less interest in long-term projects.

As far as the public was concerned, the attitude toward research on aging was, "Well, why not let the old folks die? Why do research to keep the old fossils alive?"

As for the work which had been done previous to that time, much of the work was in support of crackpot ideas, but a good deal of it revolved around the idea that if we knew enough about regeneration of organs and tissues, we could solve the problem of aging. That was a nice thesis, but it hadn't solved the problem of aging. A great deal of work had been done on regeneration in lower animals with the hope that it might throw light on the problem of aging.

But, during the past 25 years, there have been changes in the attitude of the public, there have been many changes in the attitude of those who support research, and we who are interested in the field of aging have, I think, gotten a much better conception of the aging problem even though we have not solved it yet.

I feel that the Macy Foundation has done a great deal to

most of the people here, I am interested in the field of experimental medicine. My methods have been chiefly biochemical. I have been working for some years in the field of brain metabolism and about a year ago I was asked to take charge of the research program of the Galesburg State Research Hospital at Galesburg, Illinois. Because of my interest in neurophysiology I have also been made a member of the Department of Physiology of the University of Illinois School of Medicine.

Almost all our patients are in the latter part of the life span, and a high priority in our research project is concerned with old age and mental disease of old age. My new responsibilities make me appreciate all the more the opportunity to attend this meeting

At the present time we are feeding very large doses of glutamic acid, higher than previously given in most instances, to 32 patients and to a group of normal controls who are members of the research staff I won't be able to tell you anything about the results now, because only the dietitian knows how and when glutamic acid is being given to the various subjects. In fact, we shall not begin the analysis of the results until early next summer. But I should like to say one word or two on the rationale of this experiment

Although endogenous glutamic acid is oxidized by the brain, there is fairly good evidence that it doesn't enter that organ rapidly. It penetrates the blood-brain barrier too slowly to support the rapid rate of brain metabolism.

About three years ago, Weil-Malherbe (3) suggested that glutamic acid is adrenergic and induces a prolonged, physiological stimulation of the adrenal medulla There is some evidence in favor of that possibility during hypoglycemia since his results were obtained on patients receiving insulin therapy for schizophrenia. He reported an increase of adrenaline-like substances in the blood when glutamic acid was given during this treatment Blood sugar rose, blood pressure increased and pulse rate was accelerated. That is the basis on which we started our work.

I should like to say that we are avoiding the controversial subject as to whether or not glutamic acid affects the I Q. We are also omitting all projective tests Ours is a multi-discipline attack in that we are using psychophysiological, physiological and biochemical rather than other types of tests Evaluations of the effects of our treatment upon personality structure, however, are being made by a psychiatrist.

are kept maximally in hibernation throughout their lives, their lives will be lengthened.

Two of my associates who have been studying hibernation in hamsters have initiated some work on cancer. Because it has been shown that cancers can be transferred and grown in the cheek pouches of hamsters, Dr. Charles P. Lyman and Don W Fawcett became interested in what hibernation would do to such transplants They have demonstrated that while the animals are in hibernation, the growth of the cancer transplants is inhibited However, the transplants are not completely destroyed, for as soon as hibernation is terminated, they begin to grow

Perhaps the most valuable stimulus that I have obtained from contact with this group has been to become acquainted with the atmosphere of these interesting conferences The free interchange of ideas at a very informal and relaxed level makes possible the crossing of lines between disciplines, to a degree which has not been achieved by other types of groups who have maintained their relationships at a more formal level That has so impressed some of those who have participated in Macy conferences that the pattern established here has been initiated elsewhere

EARL T. ENGLE: I think historically, that as others here, I became interested in this problem when Dr. Cowdry was organizing the first edition of his very well-known book on aging In the process of development of that, I became a contributor Then, of course, that dynamo, Korenchevsky, came over on a whirlwind tour, and after conferences over the country, and guided by the great foresight and leadership of the Macy Foundation, the aging club was started Not only has one been greatly stimulated over the years by the factual material that is presented here, but also there has grown up and continued that wonderful feeling of good fellowship, the sharing of opinion and point of view that has gone on I shall regret very much if this study group has no further sessions, but I shall certainly remember it with great pleasure all my life

CLIVE M McCAY: I am professor of nutrition at Cornell University My interest in aging goes back a quarter of a century, when I was a research fellow at Yale In one of Professor L B Mendel's seminars, we discussed the problem of retarded growth in rats I asked Professor Mendel if retarded rats would have longer spans of life He replied, "You are young,

promote this field. The Gerontological Society is an outgrowth of the Club for Research on Aging, which received support from the Macy Foundation. And the Gerontological Society, as we all know, has developed into a large organization I feel encouraged that, although aging is a complex problem and a long-term problem, we are making progress in its solution

GEORGE B. WISLOCKI: I was brought into this group at the invitation of Dr. Aub about ten or twelve years ago I must admit that up to that hour I had not taken any very active interest in gerontology or considered that I was going to. I would not put down gerontology or aging as one of the topics that has received much attention in the way of research in my laboratory.

There are two things perhaps which give me a slight claim to knowing a little about some manifestations of aging. These are, one, an interest together with Dr Aub in the growth of deer antlers and the endocrine factors which control them, and second, my interest in the growth and aging of the placentas of humans and various animals.

Deer antlers and placenta are organs which are favorable for the study of aging because they encompass their growth, maturation and involution in a relatively short time, besides which they offer an opportunity for analysis of their growth and age changes in terms of endocrine factors which regulate them.

Aside from these special phases of aging, I have given no real thought to the problem. However, I have learned a great deal about aging from listening to the experts in this conference group I have become familiar, on the one hand, with the very important practical issues of aging in the human race, including the care and guidance of the aged, their economic and social status and the illnesses to which they are subject. And, on the other hand, there are far more difficult and elusive problems of the biology of aging, involving the chemical and physiological factors which control the destiny of tissues. At these levels relatively little has so far been elucidated about growth and aging or about the related nature of cancer.

I have made several interesting contacts with members of this group I have arranged for Dr McCay and Dr Charles P. Lyman of my laboratory to study how hibernation affects aging. They are attempting to find out whether, if hamsters

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CELLULAR STRUCTURE

WARREN ANDREW
Department of Anatomy
Bowman Gray School of Medicine
Wake Forest College

I SHOULD LIKE to begin by expressing my very warm and sincere thanks to the officers of the Josiah Macy, Jr. Foundation, and to the organizers of this program, who made it possible for me to be here. I hope that I shall be able to follow the pattern, which is most desirable for this sort of conference, and, while bringing out some facts, aim particularly at the raising of questions and pointing toward the things that we do not know

I believe it is still admitted that the cell can be considered as a common denominator of living things This is in spite of the fact that there are changes in zoological theory, some contentions that Protozoa should not be considered as single-celled animals and that there are increasing numbers of examples of animals in which not all the organism is cellular, but in which a great deal of it is syncytial It is still admitted, too, in spite of the fact that we have pushed a long way into the ultramicroscopic field and have gotten so far below the cellular level that sometimes we do not recognize that the things we are looking at are actually within cells

The cells are important to us in the study of aging, it has seemed to me, because they are a common denominator, not particularly because they are small, since the fact that something is smaller than something else does not mean necessarily that it is more fundamental

If we had the problem of studying changes in cells with age, in an organism, such as, say, the sponge, in which there are only a few simple types of cells, which after dissociation can actually come together and within a short time form a whole new organism again, perhaps our studies would be less involved But, of course, we are primarily interested in man and in the other higher animals, and here we have immense aggregations of cells Somebody has estimated that there are 26 trillion cells in the human body, and I, for one, am willing to take that figure, since I have not seen a better one That is 26 million times a million cells

These cells differ very much qualitatively There are many different classes and groups of cells, which differ not only in their form and size

try it." I have spent a quarter of a century "trying it."

A second factor that intrigued me resulted from our studies of growth of trout, started in Connecticut while I was a student at Yale in 1925-27. We observed that slow growth in trout resulted in longer-lived fish. This led to a long series of studies with trout that ran from 1927 to 1943. We never learned the basic reason why slow growth in trout or any other species extends the span of life. From trout and rats, we gradually broadened our interests to include the whole field of gerontology. The most important influence in increasing our interest was the stimulus from the meetings of the Macy Foundation and acquaintance with the men brought together for these meetings.

LAWRENCE K. FRANK: My concern with the problems of aging began in 1923 when the Laura Spelman Rockefeller Memorial undertook to foster the study of child growth and development, focusing initially upon the pre-school child. This program, continued later by the Spelman Fund and General Education Board was enlarged to include the study of pregnancy and prenatal development, of infancy, of puberty and adolescence. Then in 1936 the Macy Foundation, on the initiative of Dr. Ludwig Kast, began to foster studies of aging and the aged, including support to the first systematic presentation on aging edited by Dr E. Vincent Cowdry.

It has been my great privilege as a Foundation executive to participate in the planning and financing of these ramifying studies of human growth which I believe constitute one of the most significant and fruitful fields of contemporary research and service

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FIGURE 1 Comparison of the Purkinje cells of young and old mice (A) Cells from a young adult animal, 163-days of age (B) Cells of a senile animal, 702 days of age There is a general tendency to diminution in amount of Nissl material and an *increased* staining reaction of the nuclear sap in senility Reprinted, by permission, from Andrew, W The Golgi apparatus in the nerve cells of the mouse from youth to senility *Am J Anat* 64, 351 *(1939)*

and structure, but also very much in their function, and in their physiological attributes.

A number of the persons who were first interested in changes in cells in old age studied nerve cells. Back in 1894, Hodge (1) studied the nerve cells of newborn infants, middle-aged men, and old men. He also studied the changes in the ganglion cells of the honey bee, where he also found differences with age.

When we became interested in changes in old age, we thought it would be well to start out on some ground that was already reasonably firm, and since there were papers on this subject of changes in the nervous system, we began to study the literature from that field, to see what changes could be found.

Fremont-Smith: Dr. Andrew, do you have a rough estimate of the number of cells in the human nervous system?

Andrew: No, I don't. I have seen the figure of 12 billion for the cerebral cortex.

Hoskins: The over-all number which you gave also includes the blood elements, does it not?

Andrew: Yes, all the cells of the body, but I do not know whether in that calculation the muscle fibers were figured as so many cells, according to the number of nuclei in the syncytia.

Gey: The number of cells for the whole organism has been carefully calculated by Dr Warren Lewis I have forgotten the number. I believe your first figure fits in with that

Andrew: That may well have been the source.

I began to study the cells of Purkinje in the cerebellum. This was a nice subject for study, because other workers, including Dolley (2), had studied these cells He had worked with them in the dog; Spiegel (3), in Germany, had worked with them in the guinea pig; Inukai (4) had studied them in the white rat; so I wanted to see whether their findings could be confirmed, and also, what new things could be found.

These cells, you remember, are so nicely arranged along the surface of the complicated "arbor vitae" of the cerebellum that we can study them in a fairly systematic way, and can make counts and compare one group of cells to another.

Figure 1 shows the Purkinje cells of the cerebellum in young (*A*) and old (*B*) mice. In a young mouse, we see a large amount of basophilic, or Nissl, material in the cytoplasm, and a quite clear background of the nuclear sap. In senile mice, on the other hand, mice of 702 days or more, the cytoplasm generally contains considerably less of the basophilic material, and there is a tendency to a somewhat greater staining capacity on the part of the nuclear background (5).

In mice, also, we found the presence of amitotic divisions, dumbbell-



FIGURE 2 Comparison of the cells of the semilunar (Gasserian) ganglion in young and old mice (A) Cells of a 40-day male mouse (B) Cells of a 693 day female mouse The decrease in amount of Nissl material, loss of clarity of the nuclear sap, and vacuolization of the cytoplasm are seen Reprinted, by permission of the Cambridge University Press, Publisher, from Andrew, W Cytological changes in senility in trigeminal ganglion, spinal cord and brain of mouse *J Anat* 75, 406 *(941)*

shaped nuclei in some of the Purkinje cells, in about three or four per cent of them, and then the presence of binucleated cells, which were the result of this type of division. We did not find that until we had studied the Purkinje cells for some time, although we had read about Inukai's finding of binucleate cells in the rat. Therefore, we were very pleased to be able to confirm some of these things.

Then we went on to other parts of the nervous system.

Figure 2 shows the semilunar ganglion of a young (*A*) and old (*B*) mouse, and again we see this very heavy basophilic material in the cytoplasm in the young animal, and the extremely clear nuclear background, whereas in the senile animal, we have much less of the basophilic material, and we have a nucleus with the deeper stain. Also, in general, the cells of the old mice have lighter-staining nucleoli.

Simms: How old are the animals in the younger group?

Andrew: This particular animal was only 40 days of age. The Purkinje cells which were shown before as being from a young animal were from an animal of 163 days.

Wislocki: How was this particular preparation stained?

Andrew: This was stained with cresyl violet, one of the Nissl stains, without a counterstain.

The changes in the nervous system seem to be frankly degenerative. The cells seem to be growing old themselves, we might say, and degenerating, and some of them are dying. Spiegel had made counts on the Purkinje cells in the guinea pig and Inukai in the rat, and they had found a decrease in the number of cells, which supposedly could mean only that they were degenerating and being dissolved.

Carlson: What kind of control did he have there as to number? He could not have a control in the same animal.

Andrew: Of course, he could not have litter mates, either. But he did have other guinea pigs of supposedly similar genetic constitution.

Carlson: So far as number of cells was concerned?

Andrew: Yes. He used those

Then, of course, you can measure the length of a folium in the cerebellum. It is a little easier place to count cells than somewhere else in the nervous system.

In some parts of the nervous system, then, not among the Purkinje cells, we find a peculiar relationship between the smaller glial cells and the big nerve cells when those nerve cells are degenerating. This has been known for a long time As a matter of fact, in Metchnikoff's book, he shows some figures of phagocytic cells, as he called them, consuming the higher cells of the body, in old individuals.

In poliomyelitis, we see an invasion of the phagocytic blood cells into the nervous tissue, and the nerve cell seems to melt down before

This process can also be greatly heightened in inanition, at least in the mouse In starved animals, you see this process going on.

But in the cerebral cortex, and in the ganglion, and in the spinal cord, the general fact that there are changes in the nerve cells with increasing age could be seen; and these changes did include a loss of Nissl material, which, of course, is a relative thing, changes in the nuclei and nucleoli, and increasing numbers of degenerate-appearing cells, and cells being taken care of, apparently, by these smaller glial cells

Lansing· How do the nucleoli change?

Andrew. In the majority of the nerve cells of young animals, the nucleolus looks very heavily stained. I really think that's because of the greater amount of chromatin on the periphery of the nucleolus, because the central part is still a light-staining structure.

Gey· It does present a vesicular appearance?

Andrew. Yes, if you get to the central portion itself. But there is so much more chromatin around it in the younger animals, that it is more conspicuous It seemed more conspicuous to us

While there are, then, these general changes in nerve cells, there are definite differences in different parts of the nervous system, certain things which are *characteristic* of aging in particular groups of nerve cells In the cells of Purkinje, for example, amitotic division of the nucleus is a rather characteristic thing in rodents The massing of pigment *in* cells is a rather characteristic thing for the ventral horn cells in man and in the lower animals Sometimes, almost 100 per cent of old individuals will show a large amount of pigment.

Engle· Dr Andrew, do you know anything about the nature of that pigment, or its metabolic origin?

Andrew. It has been called senile pigment, and I believe it is referred to as a lipofuchsin pigment. I think that Dr. Lansing probably knows twelve times as much about *it as I do*

[illegible]

about these pigme[illegible]

Andrew Is that the same type of pigment, would you say, *as is* found in old cardiac muscle, or old liver cells, or do you think this material in the nerve cells is different?

Lansing These materials appear histologically to be similar, although the *evidence for* their chemical similarity is lacking. There is no reason to doubt that they are similar materials

Wislocki They accumulate particularly in cells which *are rich in* lipids You find these pigments *in* the *inner* zone of the adrenal cortex and in various ovarian cells.

the attack of the phagocytic cells. Perhaps the nerve cell is already dead, and it is simply being removed. It was interesting to us to find more of this type of picture in the senile nervous system than is ever seen in the younger person.

Figure 3 shows a cell from the cerebral cortex of a 75-year-old individual, and shows a rather high degree of what is called satellitosis. A number of these satellites surround this nerve cell, which shows the rather degenerate characteristics of the nucleolus and the nucleus. We even find tiny remnants of nerve cells. We could follow by serial sections to show that they generally still include Nissl material.

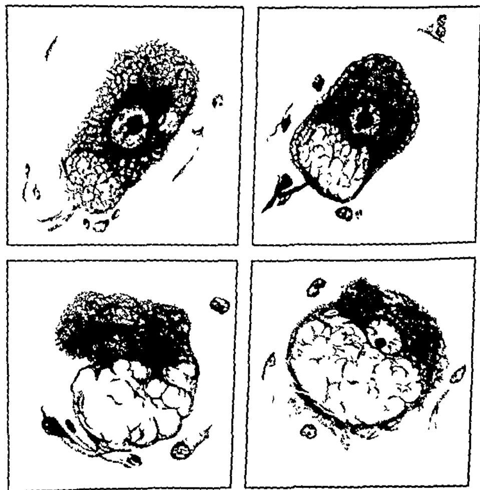


FIGURE 3 Stages in the process of fatty degeneration in the semilunar ganglion in old age. All figures from human female, 75 years old Redrawn from Truex, R. C: Morphological alterations in the gasserian ganglion cells and their association with senescence in man *Am J Path.* **16**, 255 (1940)

Wislocki: That is a very interesting question. At a previous meeting the question was brought up by McCay and Saxton as to what extent rats or hamsters really reach old age, because of the nature of the intercurrent diseases to which they are susceptible and which kill them prematurely There was, if I remember correctly, some question as to whether that type of animal ever reaches extreme old age in the sense that man does Fifty years ago, before the control of infectious diseases, the average human life expectancy was somewhere in the fifties, and a century before that, in the forties, whereas now, it is in the late sixties.

Andrew: Of course, there is still a fairly relevant question as to whether man reaches his natural span of life. He certainly leads a fairly sedentary existence, also. I doubt whether we move more than rats do

Wislocki. We might take the female, and let the reproductive period be the point The menopause in the human is around, say, 45-55 What part of the female rat's life follows the cessation of the ovarian cycles? What fraction of the total 600 days that the female of that species lives, follows the cessation of the ovarian activity?

Engle. Well, of course, the human female varies so much from all the lower animals, because the human female enters the menopause and ceases menstruation because she has no more eggs, and the ovary cannot produce hormones unless eggs are present. There is no other animal that I know of that loses all of her eggs as she comes into the last third of the span Macaque monkeys which I have studied, that have been grisly old grandmas, still have cortical ova, and go ahead with sporadic menstrual cycles at any age. That is the one respect in which the human female differs from all others

Andrew As to this matter of not being quite sure whether or not the oldest laboratory animals that we have are senile animals, I feel pretty confident, myself, that they should be described as senile, and that they are close to the end of a natural life span, or at least, relative to what we know in man, but it points up again the very sore lack of a supply of old animals kept under favorable conditions, which is such a thorn in our side in trying to work in gerontology. It certainly would not be too hard to keep animals, and under some conditions in which they would be more closely simulating a natural type of life However, instead of that, we actually do not have animals that have even been kept in cages throughout their lives. They are just not available

Hoskins Is it implicit in your presentation, up to this time, Dr Andrew, that these cytologic changes can be correlated with functional changes? Are you implying that?

Lansing: And yet, they are most prominent in cardiac muscle fibers.

Wislocki: In cardiac muscle fibers as well as in the central nervous system.

Lansing: Indeed, I think one might find them in any tissue that is studied.

Wislocki: But especially in tissues rich in lipids.

Andrew: I might interject that we almost always have thought of the pigment as chiefly in the cardiac type of muscle. At least, I did, until I read some articles on the striated skeletal muscle which showed an increase in pigment, in work done by Bucciante and Luria (6), which showed that there it is about as conspicuous a phenomenon as in the cardiac muscle

Fremont-Smith: I am struck by the statement that the pigments are highly insoluble. I cannot help wondering whether it does not suggest that the reason they are there is because they are so insoluble the body cannot get rid of them. They might conceivably be secretions of some sort, which are metabolic. This pigment is a residual which remains in the cell because it is chemically insoluble to the solvents available in the cell.

Lansing: Of course, that is really the heart of a rather old theory of aging· that if an insoluble substance forms in the cytoplasm, or indeed, in the nucleus of a cell, it cannot get out. Such materials can only accumulate with time, and theoretically, then, obstruct the vital processes It is a form of the intoxication theory of senescence.

Carlson: Dr Andrew, may I ask you: do these changes which you have described here in the Purkinje cells, and other cells in the nervous system, apply to the nerve cells in the short-lived animals, such as the mouse or the rat or the honeybee?

Andrew: I think that is one of the fascinating things about changes in the nervous system, that here we have cells that are born, we might say, with the organism. They cease their reproductive activities close to the time of birth, or, as shown in the mouse, slightly afterwards, and they live as long as the organism, if they do not undergo this degenerative change Nevertheless, the changes seen in man, who may live 90 or 100 years, and in the rat, which lives, say, three years, and in the mouse, which lives two years, are quite similar. They are the same general type of change, so that these nerve cells have those respective spans of life.

If we pause to ask the question, whether the life span of the nerve cell is conditioned by the life span, or life duration of the organism is determined by the life span of the nerve cell, we have two horns of a dilemma. I do not think that we can answer that question, but the answer to the question of whether or not the changes are similar in animals of varying life spans is certainly "yes."

been shown to have any kind of correlation at all with brain cytology.

Shock: Would not the use of tissue cultures offer a technique for circumventing some of the problems raised because of inter-relationships between different organs and tissues as they exist in the intact animal? Certainly the conditions are more readily controlled in a tissue culture. In the past, the major interest in tissue cultures has been in the structural characteristics of the cells With the many techniques now available, would it not be possible to study some of the physiological characteristics of cells grown in tissue culture? Perhaps this



FIGURE 3 Vesiculated nucleoli in living cells of a strain of human epidermoid carcinoma Explanted 8/11/51 Mag 2900 x

Andrew: I think that would be implied, yes.

Hoskins: In that case, I should like for a moment to hear Dr. Fremont-Smith's reflections on such things as Alzheimer's disease in man, which is marked by degenerative changes, and the relation of the structural changes in the brain to function.

Fremont-Smith: No, I am not the person to reflect on that, but I am sure there are others here who can. Wouldn't you do that yourself? You have given a great deal of thought to that.

Hoskins: Well, as a matter of fact, in the human situation, the correlations are not very satisfactory. One can find—I mention Alzheimer's disease as an example—people whose brains at autopsy seem to be a pretty degenerate mess and who function pretty well, and other people who function very badly without showing very much of this type of degeneration. I use this point to bring out how far we are justified in reading into what Dr Andrew is saying about implications of functional disability.

Fremont-Smith: Let me make one comment at this point, because I think this problem is too complex to try to make that kind of correlation in any specific way Just take general paresis, about which we know a great deal. Here is an individual with a very sick brain. The natural tendency has been to correlate the function of the very sick individual and his mentality with his obviously distorted and sick, dying, dead, brain cells. He has cortical atrophy, and he has atrophy of cerebral function Then we come along with malaria and pencillin —of course, previously we did have, but neglected, the spontaneous remissions—and all of a sudden, this man becomes practically normal again with respect to behavior He is functioning pretty well, and all his gross bizarre behavior distortions disappear, and yet you know that none of the cortical atrophy has disappeared Therefore, it is perfectly obvious that the functional correlation had to be made with the living cells, and that the pathology which you could see, which was quite gross, was *fundamentally unrelated to the change in function.*

Therefore, I think if we bring that question up, we are going to be lost, because the brain is a whole series of organs, and we cannot separate our functions and relate them to the organ of the brain which we are talking about.

Carlson: May I add a comment which will strengthen your comments, Dr Fremont-Smith? In certain types of so-called psychic mental disturbances, we are actually improving a certain percentage of the cases by cutting of the frontal lobe

Fremont-Smith: Exactly. It points it up very nicely.

Hoskins: It should also be pointed out that one of the most severe disturbances at the functional level, that seen in schizophrenia, has not

In exposing strains of cells to various viruses, we find that many cells are killed (9, 10, 11). These cells often *calcify* (11), *and they* remain in our cultures for long periods. This is the kind of material that was brought up a moment ago as a rather difficult thing to get rid of, and one wonders whether, in many onslaughts that man experiences in various infections and in trying to get rid of them, some of these deposits don't actually stay there for a very long time.

I can also give you evidence that certain types of injury, such as



FIGURE 6. Appearance of living roller tube culture of Rat Sarcoma 319, 48 hours after last transfer and 126 days following exposure to 1000 r of X-rays. Mag. 255 x



FIGURE 5 Vesiculated nuceoli in fixed cells of a strain of human epidermoid carcinoma Explanted 8/11 51 Mag 2900 x

approach would be of value in determining correlations between structure and function.

Gey: Dr Andrew brought up a statement or two that has made me mull over them in relationship to other things He mentioned polio virus, and I hope that we shall mention other viruses as we go along in this conference.

Variation in functional competence in cells, without interference with processes due to disease, does not seem to fit into my thinking. Man is constantly exposed to a number of ubiquitous harmful agents, and microorganisms are among them.

Now, what do some viruses do to cells? In the first place, Dr. Andrew mentioned the nucleolus In radiation injury, one sees vesiculated nucleoli. I called this to the attention of Dr Warren Lewis in the mid 1930's, and he has since reported very nice examples of vesiculated nucleoli in cultured cells (7). In fowl pox also, one sees vesiculated nucleoli (8). The virus is able to get into the nucleus rather easily.

In strain cultures of carcinoma of the cervix, we see greatly vesiculated nucleoli (Figures 4 and 5).

because at the time the photographs were taken, they were in an ordinary test tube.

ular fibroblast This is a strain that Mrs Gey has to date cultivated for well over 20 years. If one exposes these cells to 1000 r (Figure 6) and 4000 r of X-rays (Figure 7) and examines the preparation about 120

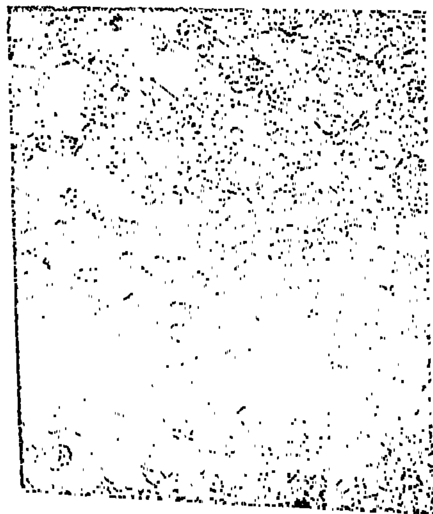


Fig 5 Roller tube culture of Walker Rat Sarcoma 319, 134 days following 1000 r of X rays Cells apparently recovered Mag 255 x

radiation injury, are extremely difficult to get rid of. If one gives sublethal doses of radiation to strains of cells and carries these cells on for months afterwards, he gets only slow recovery. In fact, not until some six months or so after he has given the radiation.

Carlson: When you say the effect of radiation, you mean the chemical residual, rather than the functional effect?

Gey: Yes, the chemical residual or, let us say, the cytopathological lesions that remain. I am not proud of these as cytological specimens,



FIGURE 7 Similar culture 48 hours following transfer and 120 days after 4000 r of X-rays Note greatly swollen vacuolated cells Culture exposed to 1000 r shows several dividing cells Mag. 255 x.

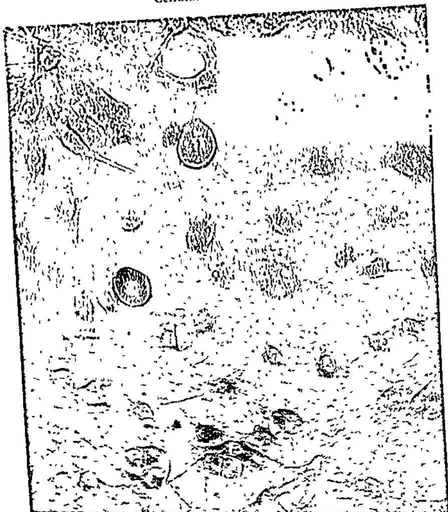


FIGURE 10 Similar culture 134 days following 4000 r of X-rays This shows a dividing cell and greatly swollen cells in this living culture Mag 255 x

1000 r, 2000 r, and 4000 r (and mind you, the maximal dosage, or lethal dosage, is somewhere around 6000 r), these cells, from 120 to 134 days following radiation, are still greatly swollen in the 2000 r and 4000 r group I must remind you that for this strain, a dose of 6000 r approximates the 100 per cent lethal dose

Fremont-Smith Are these the same cells, or are they divided and duplicated?

Gey. There is some division going on There is a dividing cell in Figure 10 There is a very slow division rate going on in these irra-

days later, one finds that the cells are still swollen from radiation injury in the 4000 r group.* This persistence of a damaged state is still present even though we have transferred them from tube to tube serially. In the case of several of these specimens (Figures 8, 9, 10, 11) given

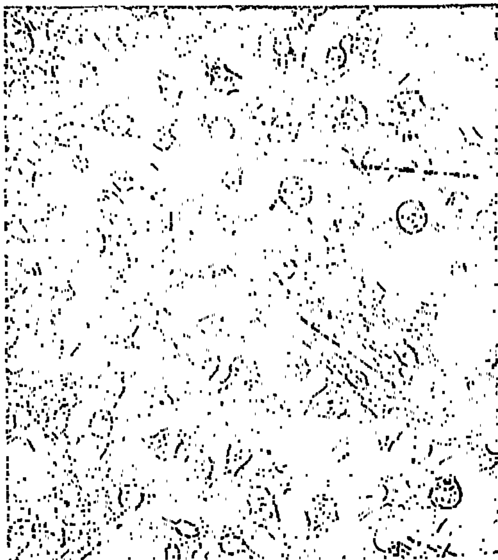


FIGURE 9 Similar culture 134 days following 2000 r of X-rays Cells slightly swollen Mag 255 x

*This study of the effects of radiation on strains of cultured cells was supported by the International Cancer Research Foundation of Philadelphia and only a few of these results were given in their annual report of 1935. The work was also supported by the Carnegie Institution, Department of Terrestrial Magnetism, and some of it was done in collaboration with Dr. Merle Tuve and Dr Larry Hafstad before World War II We hope to be able to report our complete findings at some future time.

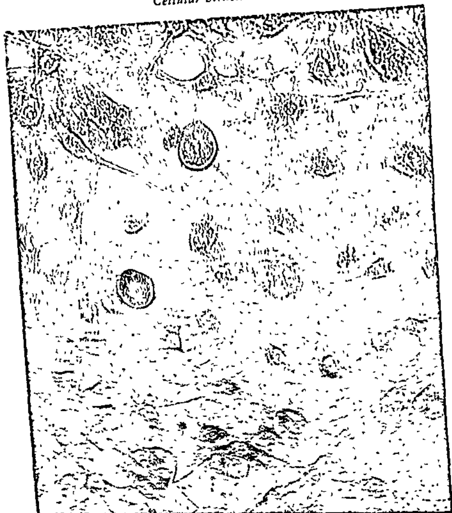


FIGURE 10 Similar culture 134 days following 4000 r of X-rays This shows a dividing cell and greatly swollen cells in this living culture Mag 255 x

1000 r, 2000 r, and 4000 r (and mind you, the maximal dosage, or lethal dosage, is somewhere around 6000 r), these cells, from 120 to 134 days following radiation, are still greatly swollen in the 2000 r and 4000 r group I must remind you that for this strain, a dose of 6000 r approximates the 100 per cent lethal dose

Fremont-Smith Are these the same cells, or are they divided and duplicated?

Gey There is some division going on There is a dividing cell in Figure 10 There is a very slow division rate going on in these irra-



FIGURE 11 Similar culture 134 days following 4000 r of X-rays Note the greatly swollen cells in this living culture Mag 255 x

diated continuous roller tube cultures even though serial renewal of medium had been practiced

This swollen condition and slow growth was observed to be present for about half a year before the entire preparation got back to "normal" and restored to us the original strain as though nothing had ever happened. However, it took a long time even under the conditions of stimulation by renewal of medium to get these cells to come back

Now, under the conditions of dormancy *that one finds in an intact* host, how long a restoration period would be required for this tissue

to get back to where it was originally? Or of a normal tissue? This is the only point that I wished to emphasize

Hoskins: Is the proliferative factor coming in?

Gey: There is very little proliferation (cell division) in these cultures. During the protracted injury period, growth is greatly retarded, as compared to the controls

Figures 6 and 7 show a low-dose experimental tube 126 days following 1000 r and a high-dose tube 120 days following 4000 r of X-rays The low dose of 1000 r compared with the 4000 r irradiated culture shows, long after the radiation, greatly swollen cells in the high dose group. These cells are full of droplets, evidently lipoprotein vacuoles which may be swollen mitochondria. They are not easily gotten rid of. They remain in these cells, no matter how good is the medium used for renewals The medium used was certainly able to keep cells in the control cultures growing progressively We had many dividing cells in the controls and low-level irradiated cultures The control grows in the order of tenfold in 10 days, a rather rapidly growing rat sarcoma. I am sure that one could explore with this kind of material and method the possibilities in terms of cumulative cell damage, regardless of what the injurious agent might be.

Lansing: Is it your point, then, that aging involves an accumulation of injuries that are products of external forces?

Gey: I would say there are two things This is one kind of thing This can be accumulative In the case of viruses, one gets also into the carrier situation where persistence of virus is a factor.

Lansing: That still is an external agent—the virus

Gey That's right.

Lansing: Are you postulating, then, that external sources of damages—

Gey What I am saying is that this picture is a most intimate relationship to the cellular pathology of aging, as I see it now These forces are the things that get right into the cell There isn't a part of a cell that I could think of that is not interfered with by a virus The damage seen is sometimes a complete dissolution, with the organism being able to get rid of all the residual products In many cases, calcified masses of material develop, which I feel pretty certain are rather difficult for the cell to get rid of

The other situation is this carrier state, which one may find in a great many viruses and polio appears to be one of these diseases

Simms You are not saying that this is aging of cells?

Gey. I am saying that the accumulation of damages with age that occurs over and over again does affect the competence of a tissue to regain its original composition, as of some period in the past

Lansing: Are you implying that in the absence of these external factors, the tissues may live indefinitely?

Gey: I should say that might be the correct answer.

Andrew: I think this emphasizes how our lack of knowledge in other fields contributes to our lack of knowledge in aging, because certainly the whole field of possible relations of viruses to cells, during what we consider their natural life span, remains not thoroughly explored.

I wanted to make one other comment about the matter of the accumulation of pigments. It is of some interest that among various groups of invertebrates, accumulation of pigments is bound up with the excretory process. We may learn more about that later during this conference; but I recall that one type of invertebrate produces certain brown bodies that seem to be just about the only excretory product they have, and these are rounded balls of excretory material that are then passed out. There are other examples of the relation of pigment to the excretory process in lower forms of life.

I was stating that in the nervous system, we have these various changes, and it is an interesting fact that in different parts of the nervous system, there are differences in the type of change I mentioned the tendency to division of the nucleus in the Purkinje cells, and then this accumulation of pigment in the ventral horn cells.

Returning to Figure 3 (p. 32), reproduced from some work by Ray Truex (13) one can see that in sensory ganglia, we have different stages of what is true fatty degeneration of single cells, where the nucleus is pushed to one side and fat is accumulating, and finally one sees a cell that is hard to recognize as such Truex has shown that there is a considerable amount of this degeneration in the sensory ganglia, but has not been able to find it in autonomic ganglia nor in other parts of the nervous system

The whole question of how marked the histological changes in different tissues are, is an interesting one and a difficult one on which to be very definite. During the course of our interest in aging, we have studied the literature and carried out projects on diverse organs, such as the thyroid, liver, pancreas, and lymphoid organs, including the lymph nodes and the spleen, and more recently, the salivary glands I know that it is sometimes asserted that the differences which we see in looking at young and old tissue under the microscope are fairly subtle, and sometimes minimal One person who has, at times, made that point is Dr. Lansing, for whose opinions on any subject I have a very profound and abiding respect. I think there is a good deal of truth in this contention, particularly in certain organs, such as the liver and kidney, in ordinary human material In general, pathologists have

not paid too much attention to differences between these organs They describe the organ of a young, or of a senile individual, and describe the pathological changes without so much reference to possible effects of aging

We have felt that in studying rather large numbers of animals, organs usually from about 50 to 100 animals of pedigreed strains, that we could come to the point where we felt that we could tell fairly well whether the organs were from young, middle-aged, or senile animals, and that there were rather definite criteria, sometimes rather subtle, and other times, very marked We have tried, in some cases, to extend this work to human tissues, usually with some result that we thought was more or less fruitful.

Gross: Dr Andrew, in relation to these subtle changes you mention, don't you feel that perhaps they are related in some way to this discrepancy between the marked pathological damage and the minimal functional disability one occasionally sees as well as the converse—the point that Dr. Hoskins and Dr Fremont-Smith discussed? Isn't it possible that the methods used at the present time are just inadequate to show up the damage that counts The subtle changes in the remaining living cells may provide the answers

Andrew Well, I very much believe in the furthering of our technical methods wherever possible and the application of them to problems of this type I think it should be occasionally pointed out that there are other deficiencies besides those of technique, and one is simply the matter of deficiency in observation of large amounts of properly controlled material. I do feel that there are still a great many things to be found by using the so-called classical methods of histology and cytology, which still, as a matter of fact, are not at all old. They have existed for a relatively few number of years

Fremont-Smith: Just in that connection, isn't our richest opportunity in the integration of the newer techniques with the so-called older techniques, rather than saying that one or the other fails? For instance this came out in [illegible] rest of the morning was spent on that, because there was absolutely no agreement at all The electronmicroscopist had his def[illegible] ba[illegible] was [illegible] on for the best definition of a biological structure should be its utility, how useful is it in giving a functional answer, an operational answer? For example, take the question of pigment which you brought up Apparently, people have felt for some time that this insoluble pigment is an important feature of the aging

process, and yet it is given the term lipofuchsin. Does lipofuchsin mean that it is a lipid that stains with fuchsin, or does it relate to the chemistry of fuchsin? To call them lipofuchsin particles, contributes little. If these things really are insoluble, as they seem to be, and the rest of the cell cytoplasm is relatively soluble, why isn't it possible to fractionate the insoluble granules, purify them, and do some chemical analyses? After all, the processes that contribute to aging and disease are chemical processes They are not matters of staining reaction.

Hoskins: I think the Land ultraviolet color-translation microscope is just begging to be used here. It was designed with the aim of correlating cytological features with the basic chemistry. Much of the spadework remains to be done in determining the appearance of the thousand and ten organic substances that will turn up in the field, but it promises to be a very sensitive instrument for this type of approach.

Wislocki: It seems to me there is one very interesting problem about the aging of cells that should be recognized, which I can present in this way: our neurons are as old as we are; we have the same set of neurons now that we had at birth, and they have not increased in number or been renewed by cell division. On the other hand, none of our hemopoietic cells have a greater life span than several months They renew themselves rapidly. Thus, the oldest blood cells which one possesses are no more than several months old, whereas the neurons correspond in age to that of the individual and may reach many decades. Therefore, the aging of a neuron is a very different thing from the aging of blood cells in terms of the time it takes and the changes involved.

On the other hand, even though the individual cells of the liver are all much younger than the corresponding neurons, the liver, as an organ, is just as old as the brain.

Therefore, it seems to me that one has to consider the aging of an organ such as the liver in which the cells renew themselves as something quite different from the aging in the central nervous system, in which the neurons do not undergo cell division and do not renew themselves

Cowdry: It seems to me you have to go further than that and consider the renewal of the chemical constituents of the nerve cells Let us say the cell is present in its original form for a long time.

Wislocki: True

Cowdry: However, it does not follow that the contents are.

Wislocki: True.

Shorr: I wonder whether agents such as tetrazolium, which throw light on the enzymatic activity of the cells, might not be applied to advantage in these studies. We have found that in the hypertensive kidney, which may appear entirely normal when studied by conven-

tional histologic techniques, tetrazolium reveals interesting abnormalities of the enzyme components.

Gey: It has been reported that antibody levels have been influenced by diet The antibody levels could readily be affected by the extent of the lipemia that exists. In this way, the nutritional state could affect the immunological state of the host from time to time.

Andreu: These are very interesting questions I think it is fascinating the way the thoughts develop, because Dr. Wislocki's statement in regard to the tissues which are composed of short-lived cells, the blood cells, was going to form a part of my summing up sentences. Now it does not matter too much whether I finish or not, because actually it has been summed up.

However, it is true that there are a lot of tissues in the body (in blood, in the bone marrow, and, of course, in the skin, the epidermis, the intestinal lining, and the lining of other tracts) in which there must be, even in old age, pretty constant renewal of cells, and the question of whether those cells are qualitatively different from the actively regenerating cells of younger individuals is certainly a worthwhile one Some work at Bowman Gray School of Medicine on blood cell counts tends to show that there is practically no difference in the white count and in the differential white count in old people from those of younger people, but there is no study of which I know on intimate qualitative characteristics of blood cells of different ages

Thuringer has shown that the epidermal cells in old skin, (at least the parts that he studied) show a higher incidence of mitotic division than they do in the young skin, so apparently there has been no letdown Of course, we do not know how long the mitotic process is taking in that older skin, but the number of mitotic divisions was definitely higher.

Shorr: Which species?

Andreu Human It was skin removed at surgical operations

Hoskins I wonder whether Dr Carlson would not care to discuss a question previously raised—how much living a starving organism actually accomplishes That has been a subject of study in his laboratory

Carlson Well, I do not have anything final on that Our most definite result seems to be injury from excess eating, rather than the analysis of undernutrition Excessive eating shortens life and favors cancer and other tumors Now, why, in the rat, moderation in eating, which gives the longest life span, should decrease cancer and other tumors, I do not know My only guess is that excess eating in addition to the weight that you are carrying, probably causes a strain on every cell in the body, but we do not know definitely. We see an example of it

where you already have, in the child, an impairment of the pancreas, the islands of Langerhans. There, without insulin, even normal eating aggravates the diabetes. Allen showed that years ago at the Rockefeller Institute, before insulin was discovered. There I saw, I believe, twenty-five children with diabetes who were undernourished, they were actually starving, but they lived relatively longer.

Hoskins: I recall the great disgust manifested in your features when you remarked at a Federation meeting, many years ago, that experiments on going without food for two weeks, I believe, resulted in a considerable amount of euphoria, a feeling that you had actually been living a little better than otherwise.

Carlson: Well, that is true Of course, I, myself, starved only seven consecutive days, but I had the longest controlled complete starvation that is on record, that is, forty-three days without food

Andrew: Was that on an experimental basis?

Carlson: Oh, yes. The man is still with me, but he is not starving now. There was one thing there that struck me peculiarly, and that was not in man, but in dogs Dr. Kunde and I had dogs which, on a given diet, kept a steady weight· no increase, no loss of weight. Then we put them on seventy-five days of complete starvation. They lost weight. Then, when we started to feed them again, giving them the same quantity and kind of food as they had had before, they not only regained their previous weight, but increased it. We had to reduce the amount of food to keep them at the old weight The starvation had trained tissue cells to utilize the food more economically.

Andrew: Well, that is the same principle that is used, of course, in repeated exercise and gaining weight as a result of supposed tissue breakdown—excessive use of the muscles I think almost any consideration of starvation, or amount of food, has to take into account the type and amount of activity that the animal or person is undergoing, as well as just the intake, because no organism is a passive intake mechanism I think it is very difficult to have the increased exercise without getting increased appetite

I thought this might be a good time to bring in a picture of the salivary glands In the salivary glands of the rat, I have found an interesting age difference, illustrated in Figure 12 The differences seem to be pretty consistent when we compare the parotid gland and the submandibular gland In the parotid gland, we found a rather marked degree of fatty degeneration, real destruction of the parenchyma, in old [illegible]

Here (Figure 12) is the parotid gland [illegible] intact parenchyma. Figure 12 A is from a rat of 302 days, and

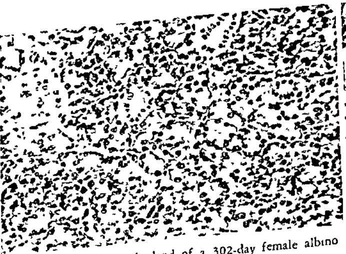


FIGURE 12 A Parotid gland of a 302-day female albino rat The parenchyma is intact

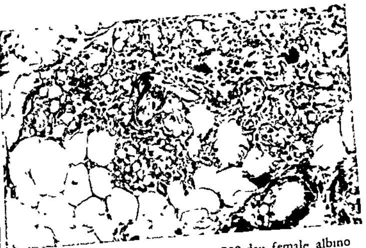


FIGURE 12 B Parotid gland of a 700-day female albino rat. Fatty degeneration and loss of normal architecture of the parenchyma are seen

Reprinted, by permission, from Andrew, W Age changes in the parotid glands of Wistar Institute rats with special reference to the occurrence of oncocytes in senility *Am J Path* 85, *157 (1949)*

Figure 12 B is from a rat of 700 days. All of the area at the left of the figure has undergone a parenchymatous type of fatty degeneration, so that only the deeply staining parts represent the remaining parenchyma.

When we examine these sections at higher magnification (Figure 13), we see that the forms of some of the alveoli are pretty well retained. The alveolus is of a pretty normal size and shape. Then the fat begins to accumulate and the alveolus is greatly expanded. We see vacuoles in the cells, merging centrally in the lumen. The thing becomes still more expanded, and finally, we have nothing left but these fatty spaces (15). (I do not want to go into the general subject of fatty degeneration. It is too complicated.)

This change, then, in the parotid gland, is very definite in the old animals, but in the submandibular gland, we have found practically no fatty degeneration.

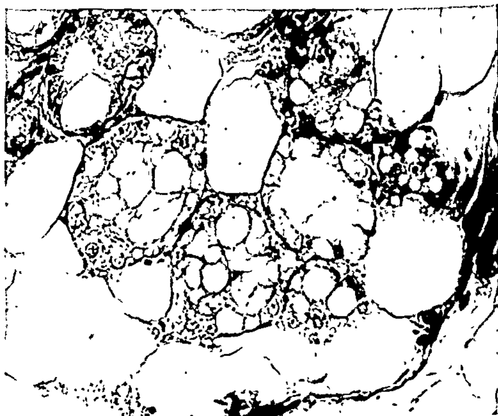


FIGURE 13 Detail of fatty degeneration in parotid gland of senile rat Several distended and altered alveoli are shown Reprinted, by permission, from Andrew, W. A comparison of age changes in salivary glands of man and of the rat *J Gerontol 7, 178 (1952)*

Engle:

Andrew The ducts are dilatec you find calculi in them It is pretty hard to recognize individual ducts in some of these areas. This, I think, is provocative—to find two glands which are rather similar microscopically (of course, the parotid, being entirely serous cells, and one portion of the submandibular gland being made up entirely of serous alveoli), and yet, to find this age change so marked in one gland, and not in the other. We thought it might be interesting to see whether oncocytes, those large aberrant cells that have been described in the salivary glands, were present in the two types of



FIGURE 14 Group of oncocytes from parotid gland of a 1000 day female Albino rat Reprinted, by permission, from Andrew, W Age changes in the parotid glands of Wistar Institute rats with special reference to the occurrence of oncocytes in senility *Am J Path* 85, 157 (1949)

salivary glands In animals of great age, we found that oncocytes were present in both types of glands. They did not seem to be correlated at all with the fatty changes.

Figure 14 shows an example of these aberrant cell nests. Normal alveoli of the parotid gland appear at the top with a group of oncocytes below them These are large cells with voluminous cytoplasm, some parts of it with richly staining basophilic material. There are also nuclear changes in the oncocytes In the center of the figure we see a clear nucleus with a tremendous nucleolus, a large body which is almost the size of some of the nuclei of normal cells.

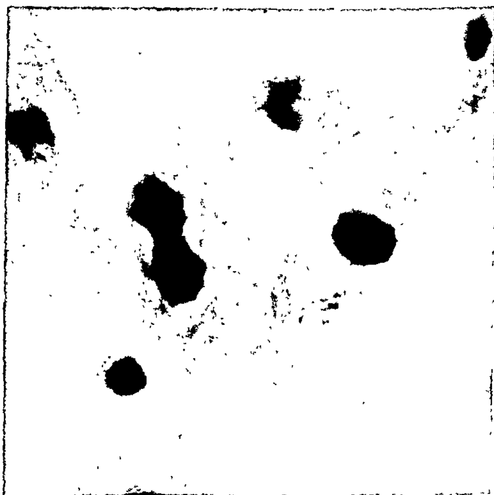


FIGURE 15 Nucleus of an oncocyte in amitotic division Reprinted, by permission, from Andrew, W Age changes in the parotid glands of Wistar Institute rats with special reference to the occurrence of oncocytes in senility *Am J Path* 85, 157 (1949)

These oncocytes, then, occur both in the submandibular and in the parotid glands

In Figure 15, we can see the type of amitotic division that occurs in them To show one picture of presumed amitosis is not too satisfactory, but there are many examples in the sections, and there does seem to be division, at least of the nucleus, in these oncocytic cells

What we want to do now is to go on and make a more detailed cytological study of the cells in these two types of glands They seem to be favorable material for studying the cytoplasmic elements Therefore, we have begun a study of the mitochondria and the Golgi apparatus. I think we are going to hear a lot about the mitochondria and the Golgi apparatus in relation to aging in the next few years Mitochondria have become prominent partly because of the fact that we have been able to learn something about their function, assigning a pretty large part of the enzymatic activity of the cell either to the content or to the surface of the mitochondria and the Golgi apparatus. While the latter is still in the throes of debate between the proponents of the artefact and of the nonartefact, it is because of that very fact, I think, that it is becoming more of a definite entity in our scientific minds. Actually, the fact that there is a reticular structure of some type, similar to w

is getting fairly

cells of the epi

lower animals has shown that whereas we used to think there was only a Golgi apparatus of scattered bodies in them, now, in the striated muscles of invertebrate animals, and in the nerve cells, a good reticular apparatus has been seen Therefore, we are learning more about the mitochondria and the Golgi apparatus In the nerve cells, there has not been a great deal of study of the cytoplasmic components, other than the Nissl bodies. This is rather surprising, in a way, because the cytoplasmic components, it seems to me, are pretty easily studied

We, ourselves, studied the Golgi apparatus (16) in the Purkinje cells, and found a change from a reticular apparatus, a very fine large net around the nucleus, in the young animals and young adults, to a rather broken down granular mass, in the senile animals Later, Dr Norman Sulkin found the same thing in the cells of the autonomic ganglia, a change from the reticular to a granular type of apparatus.

So far as I know, nobody has studied the mitochondria in nerve cells in old age. Dr Cowdry, many years ago, showed the existence of a good complement of mitochondria in the nerve cells, but they have not been studied with aging (17)

The mitochondria, of course, have fascinated many investigators for a good many years

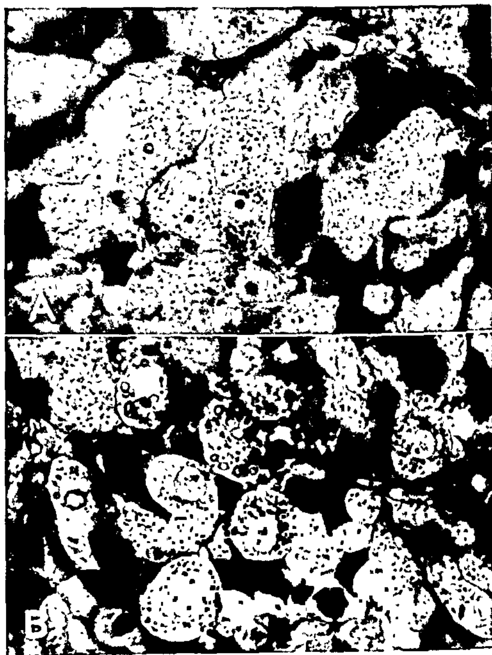


FIGURE 16 Mitochondrial changes with age in the basophiles of the pituitary gland of the fowl (A) Basophiles showing the beginning of the change, characterized by enlargement of some of the mitochondria (B) Basophiles with many vesicles in the cytoplasm, each formed by transformation of a single mitochondrium. Reprinted, by permission, from Payne, F Changes in the endocrine glands of the fowl with age *J Gerontol 4, 193 (1949)*

being bioblasts, or units of life, and since the work of Benda (19) and Meves (20) and many of the other classical investigators, who assigned so many probable or possible functions to the mitochondria.

The pioneer work on age changes in mitochondria has been done fairly recently by Fernandus Payne (21) who did some interesting work on the endocrine glands of the fowl.

Figure 16 is reproduced from his work. The basophils of the pituitary gland of a young male fowl are shown above (A), and those from a senile bird, again a male, below (B). These changes occur chiefly, or almost entirely, in the males

In the basophils of the young fowl, the mitochondria are granular homogenous structures, fairly uniform in size. In the senile fowl, many individual mitochondria have undergone a change such that they become vesicular, with a deeply staining periphery, a specific stain,

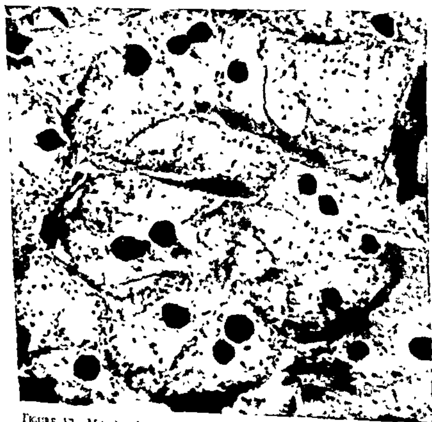


FIGURE 17 Mitochondria in the submandibular gland of a middle-aged rat, 570-days of age The mitochondrial elements are generally short, thick rods

whatever it may be, the aniline-fuchsin or the iron hematoxylin, staining the periphery, and the center remaining clear. Some of these vesicles are very large. Payne found these vesicles increasing in size and fusing to form very large bodies, with eventual destruction of individual cells due to this mitochondrial change.

We were encouraged and stimulated by hearing about this work and hearing Dr. Payne talk about it in person at one of the earlier meetings of the Gerontological Society, and we thought there was a great field for research there. Mr. Stanley Kurtz, a graduate student of mine, and I have begun some studies and published initial findings on the mitochondria and Golgi apparatus (22, 23) in the organs which I had previously studied with the more common techniques.

In Figure 17, we have a view of mitochondria in the submandibular gland. The nuclei are dark here. We see in a middle-aged adult male,

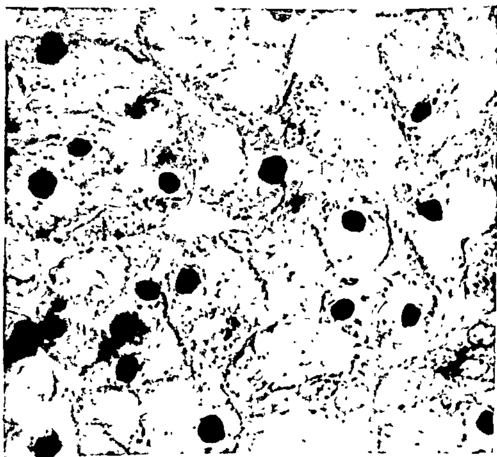


FIGURE 18 Mitochondria in the submandibular gland of a senile rat, 997-days of age. The mitochondria are chiefly tenuous filaments

a 570-day-old rat, that the mitochondria are short, thick rods, for the most part, some granules, but they have a pretty definite aspect.

In Figure 18, in a rat of 977 days, we see that the mitochondria in the submandibular gland are considerably more tenuous in appearance. There seems to be less mitochondrial material, and many of the mitochondria appear as rather drawn out filamentous structures, scattered through the cell

The mitochondria in oncocytes are not particularly different from those in ordinary alveolar cells, and the change in the mitochondrial picture in the alveolar cells in general would seem, then, to precede any change in the direction of this aberrant cell type, the oncocyte The mitochondria in the ducts seem to be less altered than do those in the alveoli

We have not seen, in the salivary glands, the vesicles that were de-

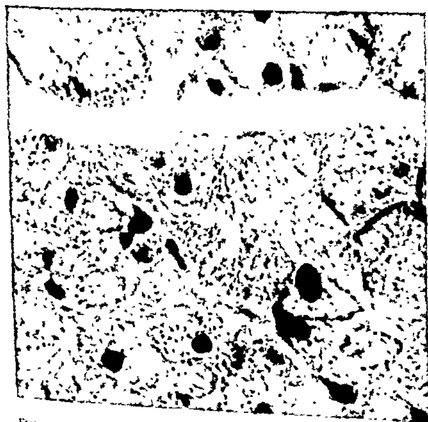


FIGURE 19 Parotid gland of a young (100-day) rat The *mitochondria* are long and filamentous

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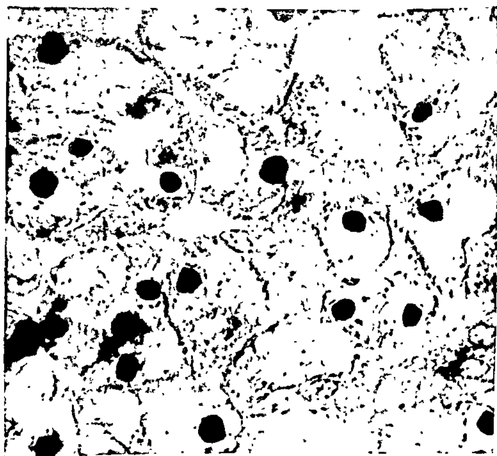


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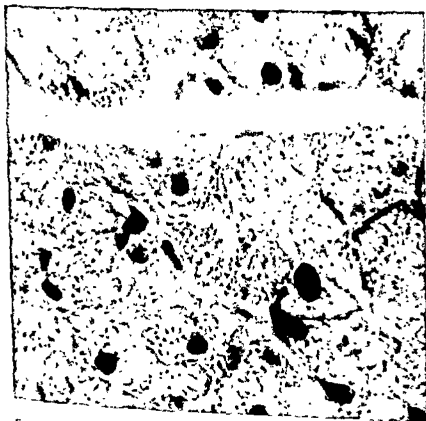


FIGURE 19 Parotid gland of a young (100-day) rat The mitochondria are long and filamentous

scribed so clearly and shown in pictures by Payne, but we do find those vesicles in the liver. In the mitochondria of the liver, we find a change to small vesicles, although nothing of the dramatic type that Payne has shown in the endocrine glands of the fowl.

Of course, the liver is an organ in which it is sometimes a little confusing to pick out the cytoplasmic elements, but we feel quite certain that we are seeing the same type of mitochondrial change, namely, the vesicular, in some of our senile livers.

Then, too, this vesicular change that Payne has described, we have been able to bring about very markedly by pilocarpine injections. There we see vesicles all through the salivary glands. The senile changes in the mitochondria of the consistently observed salivary glands, in the submandibular, are as follows: a change from the short thick rod, or coarse granule, to the finer filament, with a probable decrease in total mitochondrial material.

In the parotid gland, since we mentioned the different behavior of these glands, we were interested in seeing whether the same type of mitochondrial change would occur. We have been interested in studying that a little, but we have not gotten to the point of working out mitochondrial changes in relation to the actual fatty degeneration.

However, Figure 19 shows the young parotid gland with the mitochondria appearing as fairly small granules, and in some cases, as short rods or as granules bound together by narrower portions. That is the general picture in the parotid gland of the young animal. It is not too unlike that of the submandibular gland, and yet there is a greater paucity in definite rods, and the general aspect is somewhat different.

In the senile parotid gland (Figure 20) on the other hand, we do not have the thin filaments that we have in the submandibular gland, but in this figure we do seem to have a sort of general increase in the coarseness of the mitochondrial granules, and a greater number of the spheroidal forms. This is a 620-day-old parotid gland. We see that the mitochondria are very frequently present as spheres of pretty large size.

It is interesting to speculate whether the increase in size might be leading on toward the type of change which has been called vesicular, because one of the early changes of the mitochondria which are going to become vesicles is an increase in size, but to date, we have not seen anything that we could call vesicles in the senile parotid gland

Cowdry: Are some of your preparations stained with aniline-fuchsin, and others with iron-hematoxylin?

Andrew: Yes, we have used both types of stain We attempted to fix some of our material by perfusion, but it was not very successful

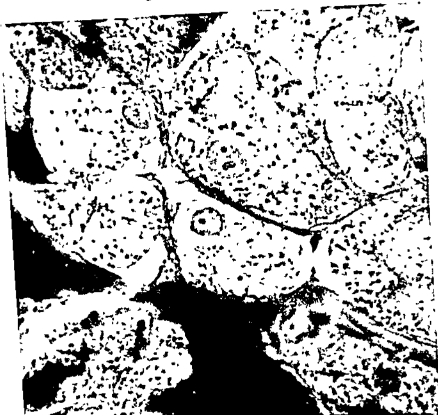


FIGURE 20 Parotid gland of an old (620-day) rat The mitochondria tend to assume speroidal form.

in the case of the salivary glands The perfusion technique did not work out well, so that we have gone back to the immersion However, we do have some material fixed by perfusion to demonstrate the Golgi apparatus, which I hope to show

Gey: Are these preparations prepared in such a way that there is no thermal shock involved? In other words, is there a decisive lowering of the temperature much below the isothermal temperature of the host? I make this comment and ask the question because if one chills cells that are thinly spread out, one gets dramatic changes in cell morphology I wondered whether the conditions under which these were prepared were the same all the way through, as far as temperature is concerned The perfusion story would be very important too.

Andrew: Actually, these vary In some of them, the fixing fluid was heated to body temperature, and in others, it was not We have not been able to see a great deal of difference in the mitochondrial picture,

although I think it probably would be a good general rule to do away with any possible effect of temperature.

These studies, the results of which we represent here in a rather simple way, were made on sixty animals at different ages. Some are Wistar Institute rats, and some of them are rats from Dr. McCay's colonies at Cornell.

Carlson: May I ask whether you have any data on any change in the quantity or quality of the saliva from these glands with age?

Andrew: We do not have data on the quantity or the quality of the saliva of the rat. There have been other studies made on human saliva, both in regard to the quantity and in regard to the ptyalin content of the saliva, and both the quantity and the relative amount of ptyalin from the parotid gland were found to be reduced in older human individuals.

Carlson: That's right

Andrew: That was shown by Meyer and Necheles, I believe, in Chicago.

I suppose we could certainly feel that the same probably is true of the rat if samples of saliva could be collected. Certainly, when one looks at a field of the parotid gland, for instance, in which some of this fatty degeneration has gone on, one would think that there was a marked change in the amount of functional activity of the gland.

We have not been very successful in trying to get pictures of secretion granules. I do not believe that anything represented here involves secretion granules, because in some cases, we have been able to see the secretion granules packed into the apical ends of the cells, and even in the human subject, we have some preparations that show the secretion granules nicely They are much larger than the mitochondria, and they have a definite localization in the apical ends of the cells. Frequently, they are surrounded by a little clear area However, our studies do not show any particular relationship between mitochondria and secretory activity They have been limited pretty much to study of the morphology of mitochondria

We have made preparations of Golgi apparatus from a number of these animals.

Wislocki: I was going to say that many cytological components which are now described merely by their shape or selective staining will ultimately be given chemical significance by the development of cytochemistry Professor Bensley, a pioneer in this field, demonstrated about twenty years ago a high phospholipid content of mitochondria, and Professor Baker of Oxford some six or seven years ago, introduced an acid hematin staining method which he regards as specific for phospholipids It has been demonstrated recently by a histochemical

reaction that mitochondria are rich in succinic dehydrogenase activity. As a result of the development of histochemical and cytochemical methods, there is some possibility of analyzing the age changes in mitochondria and other organelles in terms of specific reactions rather than in terms merely of morphological appearances.

Hoskins: Has anything been done, George, on the functional geometry of the situation? If this is an enzyme setup, then, of course, the surface exposed becomes an important factor. As the mitochondrial material conglomerates, of course, that cuts down the total surface involved, and presumably, that factor might make a good deal of difference in functional activity.

Wislocki: It has been stressed in recent years by cytologists that the surface properties and internal structure of mitochondria and other organelles are extremely complex and that many of the enzymatic activities of cells take place at such surfaces. The recent work of Palade of the Rockefeller Institute on the fine structure of mitochondria illustrates the complex internal structure of mitochondria. Electronmicroscope studies show that the surfaces of many cells are elaborately folded, indicating that extensive surface areas are necessary for some enzymatic or metabolic activities.

Andrew: The thought just occurs to me now that the apparent change here from a short thick form to a longer drawn out form, might conceivably be some adaptation for increased surface, if there were a decrease in actual amount of mitochondrial material.

Gey: When one follows the mitochondria in the living cell, one [illegible] They often [illegible] one gets the [illegible] from the [illegible] that they are actually ejected [illegible] They form [illegible] nd distorted [illegible] cystic form, [illegible] seen in cells destroyed by abnormal circumstances or by virus.

Andrew. Excuse me a moment. Would the cystic form be similar to the vesicular that we showed?

Gey: Yes, I would say, under some circumstances, that it was, although one would have to have definite proof of this in terms of the development of a specific virus infection within a cell. We have some evidence that I am sure will settle this point as will work in the future that bears upon this.

If one studies the movement of mitochondria in cells, one is impressed with the fact that the cells' hyaloplasm is perhaps the thing that kicks them about, that they do not have any independent motility

although I think it probably would be a good general rule to do away with any possible effect of temperature.

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I think they should be done in a more elaborate way in order that we get some data on how mitochondria grow.

I should like to illustrate how I think one might go about evaluating the number of mitochondria and their morphology in young and old tissues. Please keep in mind that this is not part of a study of that sort, but I think the observations point to the way one can visualize living mitochondria and follow their activity under various nutritional conditions in a cultured cell specimen

Figure 21,* shown in the dark phase, reveals many mitochondria in a human tumor cell strain (D-1 Re) You will observe that they can be easily confused with surface folds

The next image (Figure 22*) shows that no matter if we shift from

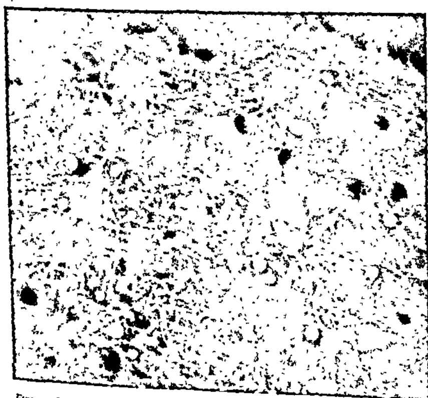


FIGURE 22 Similar portion of living cell of same type observed with bright phase microscopy Mitochondrial images predominate but surface folds also numerous Mag 3000 x

*The living cell images taken with phase microscopy (Figures 21 and 22) were prepared by by Mr Peter Sapranauskas

or show any evidence of motility. One thinks of the cristae that Dr. Palade (24) and Dr. Sjöstrand (25) have described within mitochondria. One can see how these behavior patterns (i.e., cross-sectional weakness) may be related to ultrastructure. They do show a tendency to break quickly. One rarely sees greatly thinned-out ones. Small thinned-out ones have been reported by Oberling and his group (26) in the liver after fasting. They tried to get some evidence of the origin of mitochondria. I have heard Dr. Porter speak often of his notion that perhaps the "growth granules" represent the early stages of mitochondrial development.

We have observed mitochondria for many hours under conditions of restricted nutrition in cultures, and then fed them well, and we have not seen any very dramatic changes These studies are very preliminary.

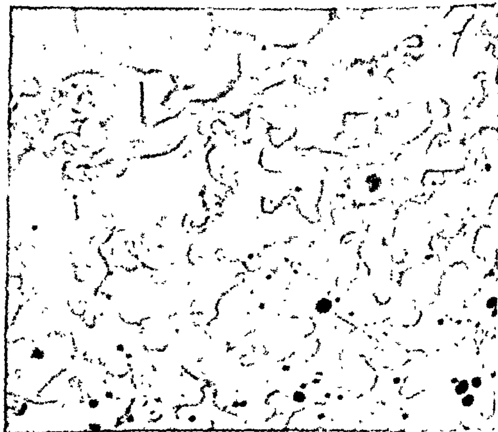


FIGURE 21 Oil immersion image with dark phase microscopy of a portion of a living human chondromyxosarcoma cell from a strain now over 20 years old. Surface folds and mitochondria difficult to evaluate Surface folds predominate. Mag. 3000 x.

that the mitochondrion might indeed arise from the cell membrane. I know I am putting my neck out, but we still get this impression when we follow the motion pictures of such images when properly timed to allow one to follow the process Such folds trap the culture medium, invaginate and produce inclusion droplets, a process known as pinocytosis.

In Figure 24 we can get some idea of the components affected by traffic within a cell. We see mitochondria, such as this little fellow, go out to the periphery and then come back in towards the great cytocenter of inclusion droplets. We have tried to get some notions of what causes all this propulsion of inclusion droplets (droplets with lipoprotein walls) and mitochondria This and the motion pictures are just a way—that's why I show this—of examining cells of different types and determining the dynamic aspects and the morphological changes that occur

Dr. Andrew mentioned something about a breastbone-type of mito-

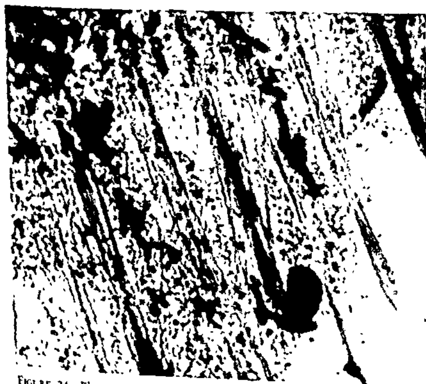


FIGURE 24 Electronmicrograph of thinned-out strain 14 p cells from a well-established strain of normal rat fibroblasts Shows fibrous strands partitioning the inclusion droplets and mitochondria Mag 18000 x.

one technique (dark phase) to another (light phase), one has difficulty in distinguishing the superficial folds from the mitochondria. Such images make it difficult to determine where some of them end. Examination of a very tiny piece of a cell, which has only one or two mitochondria in it, allows one to follow changes very carefully and to see whether, at any time, the mitochondrion disappears. We have seen small images disappear.

Shorr: What is the magnification?

Gey: This is an oil immersion preparation on the screen. It would be of the order of 3000 x, or something like that.

If one follows the undulations of the surface of this cell, one sees occasionally flat spread-out areas. When you compare these flat spread-out areas of living cells under phase microscopy with their linear aspects, which one knows are usually associated with the mitochondria of this cell, with the electron microscope images produced by Dr. Bang's laboratory, then one sees that the electron density of the mitochondrion is greater than the simple fold in the plasma cell membrane (Figure 23*) Yet, there are many situations where one gets the idea



FIGURE 23 Electronmicrograph shows folded cell membrane and other thickened areas of same cell type as Figures 9 and 10 Mag 18000 x

*Electronmicrographs, Figures 23, 24 and 25, courtesy of Dr F B Bang

Gey: These are all from living tissue cultures, and the electron-micrograph is also from a tissue culture, but it is fixed. [illegible] with buffered osmic acid, our group, [illegible] has gotten a complete localization of everything seen in the living. By such comparative studies, one gets real evidence of the lack of distortion following correct washing and fixation.

Lansing: Dr Weiss and I have been studying with the electron microscope the age changes in the pituitary gland of the mouse. We have observed that with age there is an increase in size of mitochondria, vesiculation of the latter, and disruption of their surfaces, particularly the internal surfaces This substantiates your earlier remarks, Dr. Wislocki. I have some lantern slides with me and if time permits I will be glad to show them

Fremont-Smith: We might comment, while you are getting your slides, that the movement of various particles within the cell would certainly increase the functional surface of the cell, just the way any agitation will increase the rate at which solution or chemical change will take place. This agitation within the cell *certainly* must play a role in influencing the rate of chemical reaction, by providing a greater functional surface than if everything were quiescent

Hoskins: I think that point comes out again in work at the Worcester Foundation on perfusion When the work of perfusing adrenals began, it was taken for granted that one must get the gland out of the animal and into the perfusion apparatus as nearly instantaneously as possible More recently, they find two, three, or four hours make very little difference.

Now, if you think about what must be happening to the cells in a mass without circulation, say, for five hours, I think one can assume that they are far along the way to death However, it still makes a great deal of difference how they are preserved geometrically. These presumably almost dead glands will function very well in a perfusion apparatus. I have speculated about why that is true It seems to me it is the geometrical relationships that are fairly well preserved that accounts for it.

Shorr: Are the glands kept cool in the interval?

Hoskins: It does not seem to make too much difference

Shorr: And are there no associated histological changes?

Hoskins. That, I do not know

Andrew: It is interesting that the mitochondria seem to become less independent or autonomous structures in our thinking as time passes We used to speak of the independent motion of the mitochondrion,

chondrion. We have a motion picture which shows a mitochondrion that is Y-shaped with two arms extending down two channels. Being presented with this kind of problem, what channel is it going to move down? The motion picture demonstrated that it went down both and split apart

Figure 25 shows more stress fibers within the thinly spread-out area of a cell, and you can see the mitochondria very definitely partitioned. From a motion picture, in one tiny area, one can see the mitochondria going in one direction, and in an adjoining area, one can see them going in another direction, as though there were a slip-stream between the two fluid pathways

We have not seen a mitochondrion thin out greatly. Evidently, it does not have great tensile strength Rather, we see them break across rather quickly under the various stresses of stream flow and particle pressure In this way, we can correlate their ultrastructure with their living behavior

Carlson This is in a cultured tissue?

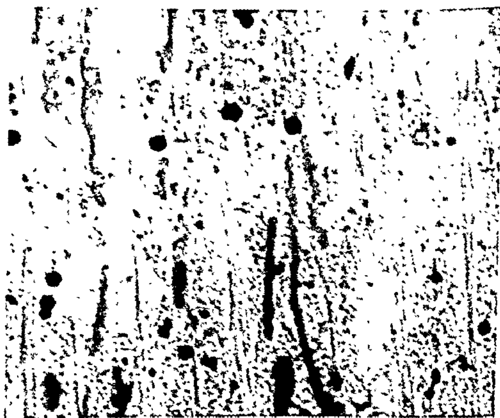


FIGURE 25 Electronmicrograph of similar cell to Figure 24 showing an area well within the free cell margin. Mag 18000 x.

Gey: These are all from living tissue cultures, and the electron-micrograph is also from a tissue culture, but it is fixed.

I might say one thing more, and that is, in setting up electron-micrographs of cells which had been observed in the living, and fixing them with buffered osmic acid, our group, working with Dr. Bang's group, has gotten a complete localization of everything seen in the living. By such comparative studies, one gets real evidence of the lack of distortion following correct washing and fixation.

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Hoskins: It does not seem to make too much difference.

Shorr: And are there no associated histological changes?

Hoskins: That, I do not know.

Andrew: It is interesting that the mitochondria seem to become less independent or autonomous structures in our thinking as time passes. We used to speak of the independent motion of the mitochondrion,

but as I hear from Dr. Gey, it seems to be due chiefly to the movements of the cytoplasm, that the mitochondria are moved about in a passive way. Of course, many of the earlier workers thought the mitochondria might reproduce themselves. There are pictures in the literature, particularly of protozoa, of mitochondria undergoing divisions at the time of cell division. However, apparently all of the studies on tissue culture, and the more recent work, tend to show there is no division of mitochondria.

Gey: Other than the work of Bernhard and Oberling (26) and of Chevremont and Frederic (27), I do not know of any recent good evidence of the growth of mitochondria and of other smaller filamentous structures (chromidies—of Hertwig and of Monné; endoplasmic reticulum—Porter) in sectioned or cultured mammalian cells up to this time. The inter-relationships of these structures and their growth through mitosis and under variations of cell nutrition are currently being investigated in several places

Cowdry: You do not find a change from these extremely small ones to large ones

Gey: That's right.

Cowdry: The presumption is that these elements are multiplied without changing their diameters Their diameters are fairly constant.

Gey. What most of us do not know, in this case, is whether the mitochondrion in the fasting animal does not get smaller and then get large again following good nourishment Most workers have no living dynamic aspect whereby they have been able to follow this stepwise and see the mitochondrion change The work of Chevremont and Frederic on mitochondrial changes during mitosis is the best evidence of change in size in living cells.

Cowdry: I was thinking of the change in volume of mitochondria from one cell to two cells, and so forth, as the cells proliferate.

Andrew: There must be a very great increase in the amount of mitochondrial material, just as there is of other material in the cell, don't you think?

Shock: Dr. Gey, you indicated that in your motion picture, you had evidence that the mitochondria break into smaller fragments

Gey: We see them break, we see them fuse We see that they are very sticky for one another, and we also see occasionally, some sort of string-like action whereby something pulls a little bud out, and when the bud is pulled out, it has nearly the same cross-sectional dimension as the main body of the mitochondrion. Somewhere else along the linear aspect of the mitochondrion, you will see another little one come out, as though something of a fibrous nature got hold of it and pulled it out.

Fremont-Smith: If it has the same diameter, that means that the total volume of the mitochondrion must have increased to make it possible for the bud to have the same diameter as the rest.

Gey: One would have to check the measurements

Fremont-Smith: That would imply, I would assume, if it took place relatively rapidly, a very rapid increase of fluid content of the mitochondrion to account for the volume increase.

Gey: You do not see very great changes in size of mitochondria, unless you shock them If you cool a cell rapidly, you can get a great glob of stuff from a mitochondrion which was formerly rather filamentous. Of course, other changes occur in mitochondria under conditions of avitaminosis, as Dr Bourne (28) has described in connection with an examination of mitochondria of cells in ascorbic deficiency. Our observations are a different situation, of course We are talking of these things that occur in a matter of minutes or hours.

Andrew: Dr. Nowinski, of the Tissue Metabolism Laboratory at the University of Texas, who is working there with Dr. Pomerat, visited our laboratory and saw these preparations and differences in the morphology of the mitochondria, and he urged me very strongly to make an attempt to get a chemist to cooperate to separate mitochondria and make studies of their enzymatic activity at different ages Of course, I think that qualitative differences in the individual mitochondria are probably better shown by working with the intact cells It might be rather difficult and complicated to study them in the isolated form, although it would be very interesting to hear whether Dr Lansing has been able to see, let us say, vesicle formation of isolated mitochondria

Of course, a lot of this work should go on with more intimate correlation among the different techniques, but it does seem to be somewhat of an order to carry on the two types of experiments at the one time and do a really god job of it I think that we might presume, from the different appearances of the mitochondria in the cells at different ages, that a study of their functional nature at different ages would be a promising field of work.

Hisaw I should think that it would be well to consider studying the giant mitochondria of insects. I was thinking particularly about Dr Carroll Williams' work (29), in which he has been able to isolate them in rather large quantities from the wing muscles of insects, and has studied them anatomically The peculiar thing about the mitochondria, he finds, is that when they do rupture, you get a ghost. That is, the original coat of the mitochondrion is left, and the contents are spilled out

I do not know whether you can see this in the mitochondria in verte-

but as I hear from Dr. Gey, it seems to be due chiefly to the movements of the cytoplasm, that the mitochondria are moved about in a passive way. Of course, many of the earlier workers thought the mitochondria might reproduce themselves. There are pictures in the literature, particularly of protozoa, of mitochondria undergoing divisions at the time of cell division. However, apparently all of the studies on tissue culture, and the more recent work, tend to show there is no division of mitochondria.

Gey: Other than the work of Bernhard and Oberling (26) and of Chevremont and Frederic (27), I do not know of any recent good evidence of the growth of mitochondria and of other smaller filamentous structures (chromidies—of Hertwig and of Monné; endoplasmic reticulum—Porter) in sectioned or cultured mammalian cells up to this time. The inter-relationships of these structures and their growth through mitosis and under variations of cell nutrition are currently being investigated in several places.

Cowdry: You do not find a change from these extremely small ones to large ones

Gey: That's right.

Cowdry: The presumption is that these elements are multiplied without changing their diameters Their diameters are fairly constant

Gey. What most of us do not know, in this case, is whether the mitochondrion in the fasting animal does not get smaller and then get large again following good nourishment Most workers have no living dynamic aspect whereby they have been able to follow this stepwise and see the mitochondrion change. The work of Chevremont and Frederic on mitochondrial changes during mitosis is the best evidence of change in size in living cells.

Cowdry. I was thinking of the change in volume of mitochondria from one cell to two cells, and so forth, as the cells proliferate.

Andrew: There must be a very great increase in the amount of mitochondrial material, just as there is of other material in the cell, don't you think?

Shock: Dr Gey, you indicated that in your motion picture, you had evidence that the mitochondria break into smaller fragments

Gey: We see them break, we see them fuse We see that they are very sticky for one another, and we also see occasionally, some sort of string-like action whereby something pulls a little bud out, and when the bud is pulled out, it has nearly the same cross-sectional dimension as the main body of the mitochondrion. Somewhere else along the linear aspect of the mitochondrion, you will see another little one come out, as though something of a fibrous nature got hold of it and pulled it out.

ng these mitochondria from the fly's muscles, with thin section methods or the electronmicroscope, and there is no evidence that they are sacs of fluid They actually have considerable detailed, fine structure

Huaw Another thing too George As a physiologist I can't quite

enzyme system in both places on an energy basis, but the end product of the metabolic mill may be different in the two cases

Wislocki: True.

Himwich: There is some evidence on mitochondria of mammals, in the liver, brain and other organs, and there are some definite ideas as to what they may be doing functionally. Not only do they carry enzymes of oxidation which make energy available but they also bear other enzymes necessary for the transfer of this energy to phosphorous compounds, a necessary step for the support of the life of the cell. In the case of the brain, glucose is oxidized and the oxidizing enzymes are probably in the mitochondria. However, it is not enough to have oxidations. The energy must be made available for the support of the structure and function of the cell In order to do so it must first be stored in energy-rich phosphate bonds as for example in phosphocreatin and adenosine triphosphate These enzyme systems are widespread within the body I therefore think your idea that they serve the same energy delivering purposes throughout the animal kingdom is correct as well as your other idea that this energy is applied differently in the various organs

Huaw: I was making it very general

Lansing I think one could also add that mitochondria in a single cell may vary Last summer Dr Hillier and I found that, after high speed centrifugation and stratification of Arbacia eggs followed by fixation and electronmicroscopic examination of thin sections, the mitochondria did not separate into a single

On the contra

in the centrifi

layer of mitoc

Apparently w ... light and heavy mitochondria which may vary in lipid content At least they manifest differences

Shorr At what

Lansing These

trifuge at 75,000 x ... 20 and 30 seconds

I should like to say that we

the dynamics of the cell, in the

of the cell I strongly believe

can run I think, particularly in studies on aging, we have to get some

brate animals. However, with insects the advantage would be that these animals live for a relatively short time, and one can test the ability of their muscles to do work at various ages. That has actually been done, to some extent. At the same time, one could study not only the morphology, but the physiology.

Andrew: I might make a general comment here. I do not know whether it is apropos or not. But in line with the matter of requests for grant money, which is a very common thing, of course, nowadays, one often feels reluctant to say that one is going to work on a butterfly, or something that low in the animal scale. I have been interested recently in noting that in the acanthocephalan worms, and in the Nematoda, there are changes in the morphology of the nuclei with increasing age of the animal, such that the very large nuclei which are present in rather constant number in the syncytial epidermis, with increasing age, tend to become first branched or budded, and then finally, to break up into rather large numbers of small nuclei in the older animals.

Perhaps I am underestimating the perspicuity of the agencies which are giving grants, but at least in relation to the government grants, it has often seemed to me a rather difficult proposition to present an application for the study of some very low form of life.

Carlson: So far as applications to government or scientific foundations are concerned, the decisions will be made by fellow scientists, and failure to receive support could come both from a lack of understanding of the fundamental problems by the advising scientists and the failure of the applicant to make clear the goal and plan of the research.

Wislocki: I should like to return to the mitochondria, if I may, and make a remark. I heard Palade deliver his paper (24), about a year ago, on the fine structure of mitochondria as revealed by the electron-microscope in rat material, and have also heard Carroll Williams deliver himself of some interesting observations on the giant mitochondria of the muscles of insect wings. I was impressed, upon hearing these descriptions, by the apparent differences in structure between the spherical fluid-containing mitochondria of insect-wings and the mitochondria provided with numerous cristae projecting into them described by Palade. I suspect, from this, that probably one should not try to come up with a general answer as to what the internal structure of a mitochondrion is. Possibly mitochondria vary considerably in structure in different species, in different situations and at different ages. That means perhaps that when we have a more complete understanding of what mitochondria are doing enzymatically, we will find that their activities differ somewhat in an insect's wing and in the parenchymal cells, say, of the liver.

Gross: Dr. Spiro, in the Biology Department at MIT, has been study-

ing these mitochondria from the fly's muscles, with thin section methods for the electronmicroscope, and there is no evidence that they are sacs of fluid. They actually have considerable detailed, fine structure.

Hisaw: Another thing, too, George. As a physiologist, I can't quite go along with you that they may be doing one thing in the wing of a fly and another thing in the liver They may be handling the same basic enzyme system in both places on an energy basis, but the end product of the metabolic mill may be different in the two cases.

Wislocki: True

Himwich: There is some evidence on mitochondria of mammals, in the liver, brain and other organs, and there are some definite ideas as to what they may be doing functionally Not only do they carry enzymes of oxidation which make energy available but they also bear other enzymes necessary for the transfer of this energy to phosphorous compounds, a necessary step for the support of the life of the cell. In the case of the brain, glucose is oxidized and the oxidizing enzymes are probably in the mitochondria However, it is not enough to have oxidations. The energy must be made available for the support of the structure and function of the cell. In order to do so it must first be stored in energy-rich phosphate bonds as for example in phosphocreatin and adenosine triphosphate These enzyme systems are widespread within the body I therefore think your idea that they serve the same energy delivering purposes throughout the animal kingdom is correct as well as your other idea that this energy is applied differently in the various organs

Hisaw: I was making it very general

Lansing: I think one could also add that mitochondria in a single cell may vary. Last summer Dr. Hillier and I found that, after high speed centrifugation and stratification of Arbacia eggs followed by fixation and electronmicroscopic examination of thin sections, the mitochondria did not separate into a single stratum as might be expected On the contrary, we found the main mass of mitochondria concentrated in the centrifugal pole of the egg near the yolk granules and a second layer of mitochondria centripetally located in the region of the oil cap. Apparently we had both light and heavy mitochondria which may vary in lipid content. At least they manifest diff-

Shorr: At what ra

Lansing: These w
trifuge at 75,000 x C ... 30 seconds

I should like to say that ...
the dynamics of the cell, in
of the cell I strongly belie
can run I think, particular ... studies on aging, we have to get some

basic data, some objective criteria, of aging, before we can begin to interpret the phenomenon at various levels. We are struggling now to find out whether an old cell is different from a young one. I believe that was essentially the terms of Dr. Andrew's discussion at the morphological level—how are old cells different from young ones.

I have been impressed over a number of years by the lack of difference that can be identified by the customary histological methods Cytologically or histologically, there is not very much that one can say is characteristic of the aging cell. When electronmicroscopic techniques became available, we thought we would try to apply this new tool to go a little further in our morphological studies. To use as a takeoff point the vesticulation of mitochondria that has been described, can we find age changes in mitochondria in the pituitary, as Payne did, and can one then go to other cells and see if that occurs as a common denominator of aging. We have not gotten that far, but we have studied the pituitary.

Figure 26 represents an electronmicrograph of mouse anterior pituitary which has been buffered in osmium, using the methods of Porter and Palade, embedded in plastic, and sectioned at somewhat less than 1/10 of a micron. The original magnification was 3,000 That 3,000 was doubled in preparing the lantern slide.

One of the first issues we came up against was the identification of the cell types in the anterior pituitary, and that is by no means simple As a matter of fact, even in the light microscope and with staining techniques, it is difficult to characterize these cell types. But as a first approximation by correlation of light microscopic examinations with electronmicroscopic examinations, I think it is reasonably safe to say that the type of cell which is characterized by a relatively empty cytoplasm, very few granules in it, and with a large nucleus is a chromophobe cell (*c*), that the cell which is very large and packed with secretory granules of uniform dimensions is an acidophil (*a*); and that a cell which has very few granules, which tends to be larger than the acidophil, although that is not apparent in this particular figure, and which is rich in endoplasmic reticulum is a basophil (*b*). The paucity of granules in the basophils and the size of the granules go along with light cytology.

Andrew: Are you speaking of all those structures as secretion granules?

Lansing: Yes, these are largely secretion granules. I believe I can show you the mitochondria in the next figure I was very much impressed with the relative lack of mitochondria in the cells of the anterior pituitary, and the fact that one sees many, many secretory granules, to the order of 20 or 30 secretory granules, to one mitochondrion.



FIGURE 26 Electronmicrograph of one-month-old mouse anterior pituitary showing acidophil (a), basophil (b), and chromophobe (c) at a magnification of approximately 8000 x Adapted from Weiss, J., and Lansing, A I Age changes in the fine structure of anterior pituitary of the mouse *Proc Soc Exper Biol & Med* 82, 460 (1953)

Figure 27 is at a somewhat higher magnification than the section I showed you before. It is also somewhat thinner than the previous section. Here we have a basophil, and here we have sections through mitochondria. Although this is not the best electronmicrograph in the world for mitochondria, one can see an outer limiting membrane and a suggestion of the cross lamination or the plates that traverse the mitochondria (*m*). This represents a one-month-old mouse. This

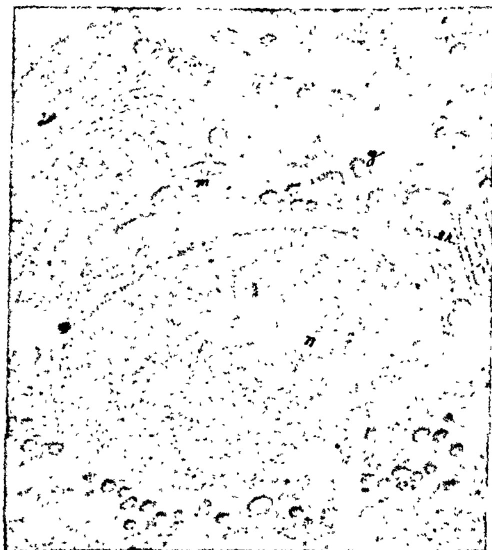


FIGURE 27. Electronmicrograph of one-month-old mouse basophil showing the typical appearance of the nucleus (n), granules (g), endoplasmic reticulum (e r.) and mitochondria (m). 12,000 x Adapted from Weiss, J, and Lansing, A I.: Age changes in the fine structure of anterior pituitary of the mouse. *Proc Soc. Exper. Biol. & Med* 82, 460 (1953).

homogeneity of internal structure is quite characteristic of a young mitochondrion. The laminations are quite dense structures, and seem to go from one lateral wall to the next. There seems to be no discontinuity The membrane, again, is quite intact

In this section, 1/10 of a micron thick, the mitochondrion is so dense that one cannot see the internal structure.

It is a reasonable guess that the mitochondrion has a high lipid content, which, of course, is not inconsistent with chemical observations.

In Figure 28, we have a somewhat higher magnification than in the preceding figure. This is a basophil of a one-year-old mouse and here



FIGURE 28 Electronmicrograph showing hypertrophied and slightly disrupted mitochondria in one year old mouse anterior pituitary 16,000 x Adapted from Weiss, J., and Lansing, A I Age changes in the fine structure of anterior pituitary of the mouse *Proc Soc Exper Biol & Med* 82, 460 (1953)

we see the fine structure of the mitochondrion even better than we did in youth. The membrane area is quite apparent, and in some spots, it almost seems to be a double-layered membrane, although I am not quite sure of that.

The internal laminations are quite apparent and appear to be double, and now they are somewhat discontinuous. This is the first indication of a difference between the young and the old. The laminations are discontinuous, whereas in the young, they are quite continuous from one lateral wall to another; and also, the mitochondria are many times larger than they are in early youth. Although there is a magnification difference here, it does not account for all of the difference in size.

Also, one may find an inclusion body that seems to have a double membrane wall and very fine particles in it. These inclusion bodies appear quite frequently in the older mitochondria.

Our findings are quite consistent with the architectural picture of mitochondria that Palade has recently described: the outer membrane and the double membraned internal shelves or plates that, of course, give tremendous internal surfaces to the mitochondria.

I might point out that the number of mitochondria does not seem to change with age. The architecture of the mitochondria conforms rather well to Payne's cytologic picture. The electronmicrographs indicate an increase in diameter of the mitochondria with age, an apparent loss of density in the medulla of the mitochondrion, and almost total loss of the transverse laminations The latter exist only as remnants attached to the outer limiting membrane.

Figure 29 illustrates the variation in size of mitochondria in aging mouse anterior pituitary The mitochondria vary from the normal young and small structures to these two to three times enlarged but otherwise apparently normal, to the vesiculated type which lacks internal structure and shows increased surface density.

Gey: With conditions of stress, when you get any hypertrophy, do you get any reversal to the other type?

Lansing: No, we have not done that We have only gone so far as to compare the very young mouse with the old mouse. There are many things that should be done now to validate these observations, but they are quite consistent with the cytological studies of Payne

Andrew: What do you consider those dark bodies to be—unaltered secretory granules?

Lansing: Unaltered secretory granules. We could find no changes in them. This, as I pointed out, is a very thin section, much thinner than the low-power picture I showed earlier

There also seem to be changes in nuclei There seems to be an increase in density of material in the cortex of the nucleus in and around



FIGURE 29 Electronmicrograph illustrating the vesiculated mitochondria with disrupted internal laminations 12,000 x Adapted from Weiss, J, and Lansing, A I Age changes in the fine structure of anterior pituitary of the mouse *Proc Soc Exper Biol & Med* 82, 460 (1953)

the nuclear membrane as if chromatin or other dense material is driven toward the very periphery of the nucleus That seems to be a very consistent change in all of the cells that we have looked at, to date.

Coudry Do you see anything that looks like virus particles?

Lansing I would not know if I saw them

Coudry Well, if they are right in saying that viruses are everywhere, you ought to see something

Lansing I am not sure Can one accept the statement that there are lurking viruses everywhere?

As you can see, in between the mitochondria and the secretory granules that we can recognize, and the nuclei that we can recognize, we have a tremendous mass of almost amorphous material, with very fine

we see the fine structure of the mitochondrion even better than we did in youth. The membrane area is quite apparent, and in some spots, it almost seems to be a double-layered membrane, although I am not quite sure of that.

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posedly simple matter of what are and are not secretion granules. In the endocrine glands, I do not recall very clear instances of the demonstration of secretion granules as such within the cells. In Payne's work, it seems as though most of the bodies which are demonstrated, as I just described, are indicated as mitochondria I just wonder what the criteria [illegible] perfectly [illegible] er, I won- [illegible] clear-cut examples of secretion granules that have been shown to be such in the endocrine glands

Hoskins: I wonder whether Dr. Gross would say anything about the possibilities of getting sections for this type of work reduced in thickness.

Gross: For what particular purpose do you mean?

Hoskins: Detailed microscopy

Gey: Thin sections

Gross: I think the question of identification is not wholly a matter of electronmicroscopy, because the electronmicroscope is only good where you have a specific label for what you are looking at.

The problem of cutting thin enough serial sections for good electronmicroscopy has been resolved Sjöstrand in Sweden is able to cut sections to a thickness of 0/01 micron, 100 Angstrom units,—and he can get measurements in these sections of 20 Angstrom units, which is about the width of two polypeptide chains. He says this is routine in his laboratory

The basic problem at this point is to know what you are looking at, to be able to say, "This is a mitochondrion," or "This is a microsome," or, "This is a secretion granule" Until you can pin the label on it, you have just accomplished a marked advance in the technique, but no real advance in understanding

One possible way of getting at this is by applying the methods of the biochemist, of fractionating out these particles, identifying them by a characteristic morphology in a relatively pure sample, showing that associated with these particles are particular functions, such as certain enzymatic activity, going back then to your sections, and finding distinctive morphology characteristic of what you have seen in your homogenates, and then trying to apply other methods to correlate the functions found in the homogenate to the particle described in the tissue This process, I think, is going to be a relatively difficult one with such things as microsomes, and not nearly so difficult with things such as mitochondria.

Andreu: I have another figure here (Figure 30) of a mitochondria preparation from the pancreas (In the pancreas in old age there seems

little granules. I do not know what they are. They may be viruses. They may be any material.

Cowdry: I should like to ask Dr. Gey: do you think any of those look like virus particles?

Gey: A lot of things in cells look like virus particles. The only way to prove that sort of thing to be a virus is to follow the progressive growth of a virus in a cell with destruction of the cell. Otherwise, it is just an impossibility.

Hoskins: What about the template size of a virus? Assuming the template theory, how small could we go and still have a functional bit of virus? Wouldn't it be submicroscopic, even with electronmicroscopy?

Gey: It would be very nearly that. It would be almost impossible to show anything in the way of a particulate thing.

Wislocki: With what technique?

Gey: With thin sectioning Attempts have been made recently, in Dr Bang's laboratory with thin sectioning and in our laboratory with motion picture studies, to visualize the changes that occur in cells when polio develops in them. According to him, they have not shown very much There are suggestive things, but polio virus is an extremely small one.

Gross: This may not be entirely a matter of size, because there is the matter of electron density.

Gey: Yes, that is another factor.

Hoskins: Taking the discussion back, for just a moment, to where Dr. Himwich left it off, the hazard of trying to translate metabolic chemistry over to mitochondrial morphology seems to be a very tremendous one I do not know how many hundred enzymes are involved in the metabolic pattern Definitely, there are hundreds, are there not?

Himwich. Oh, yes

Hoskins. So, to find a place for each of those enzymes in the mitochondrion will be very difficult

Himwich: In order to obtain a mitochondrial preparation the tissue is homogenized, i e. the cells are broken down and centrifuged In this manner it is possible to separate three fractions: one containing the nuclei, another with mitochondria and a third with the soluble portion, the so-called microsome fraction. All the enzymatic reactions of the cells probably are to be observed in one or another of these three fractions For example, the microsome portion contains the enzyme system necessary for glycolysis.

Hoskins: Glycolysis itself is a movable function.

Himwich: Yes. Yet the whole group is in the microsome section.

Andrew: It seems to me it is a little bit difficult to analyze this sup-

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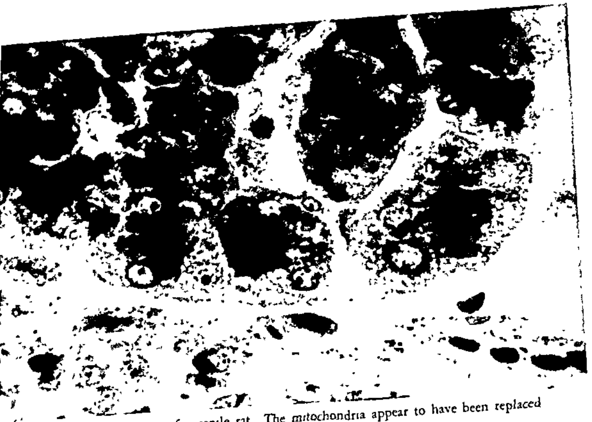


FIGURE 31 Pancreas of a senile rat The *mitochondria* appear to have been replaced by vesicles

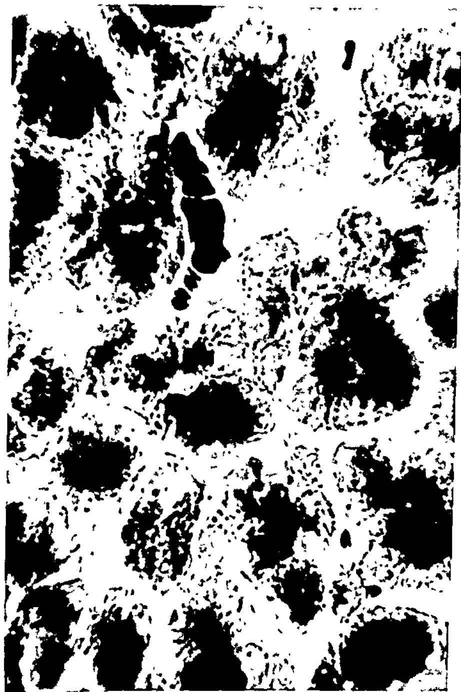


FIGURE 30. Pancreas of a young rat, with the mitochondria as well-shaped rods.

obtained by comparing photographs of sections taken at the highest magnifications of the light microscope with electronmicroscopic pictures obtained at the same magnifications.

Cowdry I think the fundamental thing is that you never study, with the electronmicroscope, a living cell or particle of a cell. It dies in the process of preparation Therefore, you have somehow to bridge the gap between the living and the dead

Wislocki. Dr. Gey said that in his tissue cultures, studied with the electronmicroscope, he had identified the very same areas seen previously in the living cells with the light microscope. He has a very fine opportunity, therefore, to observe to what degree artefacts have developed in the preparations subjected to examination in the electronmicroscope

Engle No, Vincent, what I think we have to do is to learn to in-

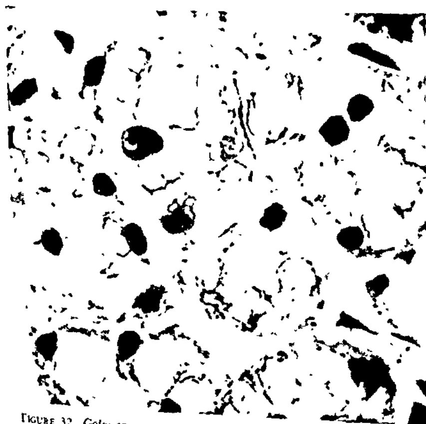


FIGURE 32 Golgi apparatus in the submandibular gland of a young rat The apparatus appears a large, continuous reticulum in these cells Perfusion fixation, Aoyama's method

to be quite a bit of localized change, groups of alveoli which show some mitochondrial change.) This is a young animal, and the secretion granules are closely packed in the apical ends of the cells toward the center of the alveoli. We can see the mitochondria as rod-shaped forms, some granules, more in the basal ends of the cells.

Figure 31 is a similar preparation from an old animal. In local areas, in the pancreas, I feel that we are able to see vesicle formation from mitochondria.

We looked at these things for quite a while before thinking that they were mitochondria However, after going through the work of Payne again, we concluded that they were. You can see the dark-staining periphery and the light-staining center, although many of them become so light that they look like vacuoles.

I believe Dr Cowdry mentioned that in the pancreas in phosphorous poisoning, you get very large mitochondria.

Cowdry: They don't look like that. I wouldn't know whether a preparation like that gave a positive Feulgen reaction or not. They look to me as though they would not

Andrew: Well, we don't have a Feulgen reaction on this Do you think that they would be, actually?

Cowdry I don't know They don't look to me like lipoid vesicles, or anything I have ever seen before Perhaps they are micronuclei. There is a dark spot in one place in most of them, which is faintly suggestive of a micronucleus.

Andrew: There is darkness on the periphery.

Gey: One can get micronuclei without so-called nucleoli associated with them

Cowdry. Am I looking at what you are? I mean those small vesicular things with very clear insides, except for a dark mass usually applied to the periphery Is that what you are referring to?

Andrew: Yes, that's it

Engle· Dr Hoskins, don't you see that we are perplexed with a problem which is essentially one of semantics? From time to time, I am called down to the electronmicroscope lab, and I see structures which seem to have meaning to me, but I am not able to converse about them because I am not able to convert my terminology, just as Dr. Cowdry is not able to convert his terminology, to meet a new situation that has arisen It is one of those things that is going to take quite a number of years, before we can translate light microscope language to phase microscope language, to ultramicroscope language, and to electronmicroscope language. I do not think there is anything to do except to have greater frequency of interaction with these various disciplines

Wislocki: I think that important cytological information can be

tioned, the future of electronmicroscopy in biology is certainly not tied up entirely with the sectioning technique or with the extension of cytology. This is just one phase of the use of the electronmicroscope, and this is a morphological phase. It is hoped that as you get finer morphological detail, you are getting closer to the important chemistry involved

However, the electronmicroscope can also be used for the study of reactivity, not by studying complex tissues, but by studying the colloidal reaction of simple tissue constituents, components of tissues, and slowly building up in complexity to a higher level. By slowly building up at various levels, you can correlate the higher-range more complex systems with their purified components

Andrew: The next two figures illustrate some changes in the Golgi apparatus in the salivary glands which we have been studying Figure 32 is the salivary gland of a submandibular gland from a rat 100 days old. In the Golgi apparatus, it is rather interesting to note, the cells were fixed by perfusion fixation, and yet, the reticulum is seen. This was done with a silver impregnation of the Golgi apparatus, which appears as a widespread, rather delicate, reticulum in the cells of the gland of the young rat

In Figure 33, we see in an animal 900 days of age, a picture in which the threads appear considerably coarser, and in which there is a tendency for the net to be broken into individual parts in the different cells, not a fragmentation to a granular state, such as has been described for some types of nerve cells, but yet, some tendency to a definite coarsening of the reticulum with some breakdown into individual fragments of reticulum This seems to be a pretty consistent picture in both types of salivary gland, the submandibular and the parotid gland

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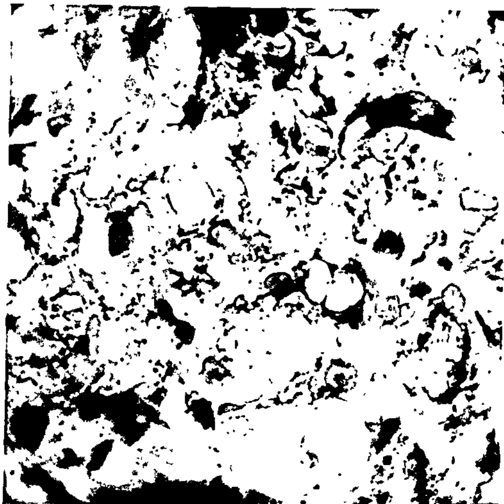


FIGURE 33 Golgi apparatus in the submandibular gland of a senile rat The apparatus is coarser and appears partially fragmented in a number of cells. Perfusion fixation, Aoyama's method

terpret artefacts. With our hematoxylin-eosin slide in our light microscope, we are looking at an artefact, but we have long learned to accept what we understand is the meaning of that artefact. Now, when we go to the electronmicroscope, we are seeing desiccated tissue which has a reasonable moiety of artefacts We have just got to translate our interpretation of artefacts under the light microscope to the interpretation of artefacts under the electronmicroscope

Cowdry: I think that is perfectly clear. I think the stamp of identification on these things, as Dr. Gross mentioned, is important, and sometimes easy, and when they are once identified, one can proceed to a fuller appreciation of structure than ever before.

Gross: I think it is worth pointing out that, as Dr. Wislocki men-

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SOME BIOCHEMICAL STUDIES ON THE PROCESS OF AGING

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I AM GREATLY honored for being invited to meet with this distinguished group, whose members have been so actively and directly involved in investigations on the process of aging. I am only a novice in this field, although for a number of years I have been interested in gerontology and approached the periphery of some aspects of the problem by comparing, qualitatively and quantitatively, urinary excretions of a number of metabolic products or other essential constituents of our body, such as individual amino acids, inorganic ions and certain water soluble vitamins, by animals of different ages. These studies were made to explore the assumption that the requirements, as estimated from retention measurements for these components may differ significantly with age. While some differences in the urinary excretion of certain amino acids were observed between young and old rats, it was difficult to attribute such findings to age alone. This empirical type of research was, therefore, not pursued further However, during other phases of our nutritional studies, we made certain observations which seemed to require further examination as possible new approaches to the problem of aging. My discussion will therefore center around these results and will be divided into two parts.

THE NUTRITIONAL ASPECTS OF GROWTH

Before talking about the process of aging, perhaps it is important to ask ourselves what are some of the biochemical and nutritional aspects of growth? The classical studies of Rose (1), Mitchell (2) and others (3, 4, 5) indicate that the protein requirements growth for can be considered adequately met if the diet contains sufficient and balanced amounts of the essential amino acids Such a diet can produce weight increase and maintain nitrogen balance. The separation of naturally occurring amino acids into essential and non-essential categories based on whether one finds a requirement for an exogenous

source for a given species and function might be misleading because the so called non-essential amino acids also play important roles in nutrition We have recently found that the dietary proteins can influence the synthesis of tissue proteins to a far greater extent than would be guessed from their content of amino acids. Experiments with dogs and rats in support of this thesis may now be summarized:

In one study on oral feeding of protein depleted dogs it was found that casein (6) or its enzymatic hydrolysate resulted in the regeneration of serum albumin and globulins (Table I), whereas lactalbumin (7) or its enzymatic hydrolysate brought about the production of serum albumin alone (Table II) On the other hand, if a minute amount of [illegible] from the casein hydrolysate by alcohol precipitation [illegible] imu- [illegible] mon- [illegible] otein depleted animals under the influence of [illegible] ietary nitrogen sources. In another study, the effect of feeding milk proteins on the synthesis of the plasma proteins of normal dogs was observed It was found that when a group of twelve adult dogs were brought to as uniform a state of nutrition as possible by feeding them a diet containing a mixture of proteins such as meat, milk proteins, and vegetable proteins in addition to the other nutritionally important ingredients such as vitamins, minerals, fat, etc , the albumin (A) to globulin (G) ratio, determined electrophoretically, ranged from 1 2 to 1 5. These animals were randomly divided into two groups of six animals

TABLE I

The Effect of Oral Feeding of Casein Hydrolysate on the Total Circulating Albumin and Globulins of Dogs Depleted in Proteins

	Albumin Per Cent ± Standard Error	Globulin Per Cent ± Standard Error
Control	100	100
Depleted	29 2 ± 2 1	102 ± 4 7
1st Period	44 5 ± 5 6	107 3 ± 11.5
2nd Period	65 2 ± 4 4	121 5 ± 10 6
3rd Period	79 0 ± 7 5	113 0 ± 10 7
4th Period	82 6 ± 8 3	126 4 ± 9 6
5th Period	85 4 ± 9 8	131 0 ± 9 6
6th Period	95 4 ± 10 2	136 2 ± 9 1

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TABLE II

The Effect of Oral Feeding of Lactalbumin Hydrolysate on the Total Circulating Albumin and Globulins of Dogs Depleted in Proteins

	Albumin	Globulin
	Per Cent ± Standard Error	Per Cent ± Standard Error
Control	100	100
Depleted	24.3 ± 2.3	93.8 ± 2.8
1st Period	39.3 ± 6.3	113 ± 5.6
2nd Period	55.8 ± 5.2	108.4 ± 5.9
3rd Period	72 ± 6.0	97.3 ± 6.9
4th Period	88 ± 7.7	91.7 ± 7.1
5th Period	88 ± 7.6	91.5 ± 5.3
6th Period	98.5 ± 7.3	101.7 ± 3.2
7th Period	104.4 ± 7.6	96 ± 4.8

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each. One group was fed casein for six months. The average A/G ratio in this group decreased to approximately 0.8 although the mean total plasma proteins concentration increased slightly (from 7.2 to 7.5 grams percent). During the next six months these dogs were fed lactalbumin. At the end of that time the average A/G ratio had risen to 1.5. Similarly, the second group of animals were first fed lactalbumin and subsequently casein during the two six-month periods. The mean A/G ratio was 1.4 after the lactalbumin feeding period, but was lowered to 1.0 by the end of the casein feeding period. These data suggest that the synthesis of plasma proteins by healthy or protein depleted dogs is dictated not so much by the amino acids fed but by some unknown accessory factor in dietary protein, since both casein and lactalbumin are universally found to be nutritionally adequate by the usual criteria of growth and maintenance of nitrogen balance.

In a series of studies, 23-day-old weanling female rats were fed diets containing different proteins (each 20 per cent of the diet) as found in meat, casein, soybean or wheat gluten. It was found that the growth rates, except of the animals fed wheat gluten, were essentially the same. However, the plasma cholinesterase content (Table III) varied with the protein component fed; those fed beef muscle having the highest value (9). These data indicate that growth rates of rats do not necessarily reflect nutritional status at least as far as the concentration of this one important enzyme is concerned, and, further, that dietary proteins, besides being a source of amino acids for growth and

TABLE III

Cholinesterase Activity of Female Rats

Diets	Total Gain in Body Weight*	Cholinesterase Activity RS Units/0 3 ml serum†
Wheat gluten	75 ± 3 6	0.60 ± .04
Soybean	161 ± 7 5	1 18 ± .11
Casein	165 ± 6 2	1 31 ± 07
Beef muscle	170 ± 4 2	1 88 ± 07

*Grams after 150 days of feeding †Mean of 10 animals

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nitrogen balance, play a directive role in the synthesis of enzymes. Collateral studies to ascertain whether plasma concentrations of other enzymes showed similar variation with diet have failed so far to establish any definite correlation.

These preliminary data indicate that a particular dietary protein can stimulate the production of one enzyme but not necessarily others To put it in another way, dietary regimen can influence the ultimate compositions of our bodies in terms of minute but physiologically important components such as enzymes and plasma protein fractions Such differences are not detectable by balance studies using the classical *measurements* involving determinations of general classes of compounds rather than specific substances like plasma protein components or enzymes

The importance of dietary protein in the synthesis of tissue proteins is also illustrated in the regeneration of hemoglobin by rats made anemic due to iron deficiency Whole milk as the sole source of food was fed to sixteen adult rats whose anemia was made more severe by removal of blood by cardiac puncture shortly before the experiment These animals were divided into four groups (two males and two females each) The groups were offered different diets containing various amounts of proteins Each diet except the protein free diet contained the same percentage of protein (25 per cent), carbohydrate (40 per cent) and fat (28 per cent) and was supplemented with the usual synthetic vitamins and minerals Each rat was fed daily by stomach tube 1 0 ml of a radiactive Fe^{59} solution (0 25 mg Fe per ml) for two weeks. Heparinized blood samples were obtained from these animals by cardiac puncture and centrifuged for the separation of blood

TABLE IV

Dietary Proteins and Ratio of Fe^{59} to Hemoglobin Regeneration

Group	Diet	Δ Body Wt. (gm.)	+Hb (gm. %)	Fe^{59} (c.p.m.)	Fe^{59}/Hb
A	Whole milk	−1	5.2	12700	2440
B	Casein	+20	8.3	15300	1840
C	Soybean	+15	5.4	7500	1390
D	Protein free	−5	4.0	9700	2420

cells from plasma. Blood forming organs such as liver and spleen were also removed for the determination of radioactivity due to Fe^{59}, after wet combustion of the tissue. It was found that there was a negligible amount of radioactivity in each ml. of plasma. The radioactivity measurements on the erythrocytes of animals in the several groups are given in Table IV. The point of particular interest to us today is that the ratio of total Fe^{59} in the cells to hemoglobin concentration varied about two fold, depending on the dietary intake. Such data again show the influence of the dietary proteins on the uptake of Fe^{59} in the formation of hemoglobin by anemic rats.

The maintenance of a normal life, biochemically speaking, may be considered as a series of enzymatic reactions. In biological aging, which may often be accelerated by stress, there can be, relatively speaking, either an over-abundance or under-production of certain tissue enzymes or proteins and concomitantly an over-all imbalance of the necessary nutrients or metabolites for subsequent series of reactions to maintain health. That such reactions can be effected by stress is demonstrated by the results of the following two studies (10).

Two groups of six male rats each were used for this study. In group A, each rat received a daily subcutaneous injection of 5 mg. of cortisone for two weeks. Each animal in the second group (B) which served as control was given a daily injection of a normal saline solution. During the experimental period, each animal received 10 gm. of stock diet per day, to insure that the observations made would not be reflections of differences in food intake. After 10 days the animals were sacrificed and the arginase (11) and amylase (12) contents of the livers were determined. The results are given in Table V. Treatment with cortisone brought about a marked decrease in body weight, but an increase in both the weight and the total nitrogen content of the liver. The arginase activity in the livers of the treated animals was higher than that of the controls whether expressed as units per gram of tissue or per organ. On the other hand, the injection of cortisone resulted in

TABLE V

The Effect of Cortisone on Enzyme Concentrations (Restricted Feeding)

	Group A Cortisone Treated	Group B Control
Body weight (gm.)		
Start	326 ± 21	330 ± 16
Necropsy	291 ± 17	367 ± 15
Liver		
Weight (gm)	16 0 ± 1 2	11 1 ± 3
Total N (mg)	392 ± 23	326 ± 19
Amylase		
μ/gm tissue	1 65 ± .12	1 61 ± 16
Total μ	26 4 ± 2 0	17 6 ± 1.9
Arginase		
μ/gm tissue	14 5 ± 1 1	8 3 ± 1 0
Total μ	233 ± 25	91 4 ± 9 7

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an increase in total amylase activity in the liver but not in activity per gram of tissue

In still another experiment (10), the effect of cortisone on plasma cholinesterase was also studied. Animals were fed casein-containing diets with or without liver supplementation One half of the animals in each group were given 5 mg of cortisone daily. It can be seen (Table VI) that animals on both diets which received cortisone lost weight and possessed a lower concentration of plasma cholinesterase. Desiccated liver was used as supplement since Erschoff (13) found it to be effective in correcting the growth retardation effect of cortisone on young weanling rats However, liver did not prevent the decrease in cholinesterase content or weight loss of these adult rats

The effect of manition, such as starvation or protein deprivation, on enzyme activity, blood clotting and complement titre was also studied The data, like those of other investigators (14), demonstrate that starvation brought about a decrease in certain enzymes, such as cholinesterase, phosphatases, and others. However, manition did not bring about a decrease in certain other physiologically important systems,

TABLE VI

The Effect of Cortisone on Plasma Cholinesterase of Rats Fed Different Diets

	32% Casein		12% Liver	
	Change in Body Wt. (gm)	Change in Serum Cholinesterase RS units/0.3 ml	Change in Body Wt. (gm)	Change in Serum Cholinesterase RS units/0.3 ml.
Controls	+7	−0 14	+7	−0 03
Cortisone Treated	−19	−0 77	−15	−0 74

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such as complement titre and blood clotting time. It is conceivable that during inanition selective destruction of certain enzyme proteins occurs readily while other protein systems, more essential for survival, are maintained.

How are these observations related to aging? It seems reasonable to regard the process of aging, in part at least, as being influenced by the cumulative effects of stress and other conditions which disturb enzyme production and result in an imbalance among enzyme systems, i e the diminution of some enzyme activities and enhancement of others. Assuming the validity of the hypothesis that enzymes, in the last analysis, must play an important role in aging we can proceed to ask ourselves the next question, namely, can the stress-induced enzyme changes always be reversed upon the removal of stress? A partial answer to this question may be found in an experiment in which healthy adult dogs were plasmapheresed every other day for two weeks, with the removal of one third of the calculated total circulating plasma proteins. It was found that after the first several plasmaphereses, there was a marked decrease in total circulating albumin and globulins except the alpha globulin which remained essentially constant Feeding animals in such a state of protein depletion with a good protein such as casein, egg white, etc, put the animals promptly in positive nitrogen balance and the plasma proteins returned to the initial levels However, if plasmapheresis were continued for such a length of time as to deplete protein reserves even more severely, as indicated by sharp decrease in the total circulating alpha globulins, the animals could not maintain nitrogen balance with an otherwise adequate casein hydroly-

sate and ultimately died. These data then demonstrate that the ability of the animal to recuperate after repeated applications of stress is limited, and in this type of experiment, the decrease in alpha globulins is a warning that the limit has been reached, and the stress effects have become irreversible

The second approach of interest involves the determination of some protein components in the organs of young and old animals, through their ability to combine with vitamin B_{12} It was found by means of vitamin B_{12} tagged with radioactive Co^{60} that this vitamin can combine *in vitro* with a number of substances such as nucleoproteins, yeast nucleic acid, etc (15). The union is of such a nature that B_{12} cannot be dissociated from the formed complex by dialysis Experiments *in vivo* show that after parenteral administration of radioactive B_{12} to young and old rats as much as 20 per cent of the injected radioactivity can be found in the kidneys shortly after injection (16). Furthermore, the rate of disappearance of radioactivity from this tissue is much more rapid than from other tissues. This disappearance rate is also dependent on the age of the animals, being faster for the older rats These latter differences may be regarded as a reflection of the presence of varying amounts of B_{12} binding proteins in the kidneys of rats of different ages That such differences exist was substantiated by the results of an experiment in which kidneys from young and old rats previously injected with radioactive B_{12} were homogenized and suspended in a 1 per cent saline solution After adjustment to pH 5 3 the suspension was centrifuged and the radioactivity in the supernatant fluid and the precipitate was determined. The results (Table VII) demonstrate that the organ of the old rats retained more radioactivity than that of the young ones, in the fraction insoluble at pH 5 3 In another series of experiments it was found that animals which were previously treated with cortisone excreted about twice as much injected radioactive vitamin B_{12} in the urine as did the controls Determinations of radioactivity

TABLE VII

Distribution of Radio-active B_{12} (in c.p m.) in Kidneys of Young and Old Rats

Days after Injection	Young		Old	
	2 Days	14 Days	2 Days	14 Days
Precipitate	240	200	410	350
Supernatant	320	20	190	10
Total	560	220	600	360

TABLE VIII

Effect of Cortisone on Radio-active B_{12} (in c.p.m.) Binding Power

Group	Treatment	Rats	B_{12} Bound Per Gram Tissue			
			Muscle	Large Intestines	Heart	Spleen
A	Saline injection	Young	8 3	62	41	40
B	Cortisone treated	Young	5.2	43	24	47
C	Saline injection	Old	5.1	38	21	41

in the tissues of treated and control animals (Table VIII) show that ability of the muscles, etc., to hold on to B_{12} has been altered following cortisone administration It is interesting to note that in preliminary studies these organs in the old rats showed a similar decrease in the B_{12} binding power. Such parallelism should not be taken to infer that aging is due to oversecretion of adrenocortical hormones, such as cortisone. As a matter of fact, it was found that at least one enzyme in plasma, cholinesterase, showed a decrease with cortisone treatment but not with aging. These results are presented to demonstrate a unique way of following changes in the chemical composition of the body under the influence of aging and stress or other conditions

In conclusion it may be stated that the data presented at this time demonstrate in the first place, that dietary proteins can influence the body composition of growing rats or of healthy or protein depleted adult dogs, so far as enzymes and plasma protein components are concerned. The concentration of some enzymes can be shifted when stress is applied and the imbalance, in some instances, at least, can be corrected by dietary means. It is conceivable then that the process of aging, often accompanied by stress, can also result in the imbalance of some enzymes. It is therefore of prime interest to establish the role which diet plays in maintaining a balanced enzyme system An interesting method of determination of a chemical substance or substances in tissue by estimating the combining power with a radioactive substance like vitamin B_{12} tagged with Co^{60} has been presented Such studies revealed differences in the composition of the young and old tissues

Lansing: This is just to assume, though, at this time that enzyme changes are involved in aging. What evidence have we that this is so rather than other arbitrary biologic components? Nuclear membranes may be involved in aging, or x, y, or z I think that you begin with an arbitrary assumption here

Chow: Not necessarily What we want to do is to find out whether there are age changes in enzyme systems.

Lansing: It may well be that the enzyme systems are quite unchanged through the aging processes, that they are very much intact, that the oxidative enzymes are right on the nose in regard to qualitative and quantitative aspects of their dynamics, but there may be a structural change, or some other change in the cell that is responsible for aging.

Chow: That may be true, but the investigation of enzyme systems is just as legitimate as the investigation of minute structure.

Andrew: I think Dr Chow's point that we find it very difficult to find any organism with uncomplicated aging is a good one. On the other hand, I do not think that should be overemphasized because it is true of practically anything else in which we may think we can't find a patient who has scarlet fever and has no other deviations from the average Everything must be expressed in terms of variables, and I think that old age is about as definite and consistent a thing as many other conditions which we must study and in which we must take into account the other variable factors

Simms: You just raised the question as to what is aging, and I think it might be well to adopt some arbitrary definition of aging at this point as a basis for our discussion We have to remember that aging has many different manifestations, but one which is of interest to almost everyone is the effect of aging on the life span and on the mortality rate. The mortality rate increases with advancing age because of the fact that the accumulation of pathological lesions increases with advancing age If you are willing to accept that as the aspect of aging that is under discussion, then it would be important to show that if there are changes in the enzyme systems with advancing age, that those changes affect the rate of accumulation of lesions

Shock: Dr Himwich, won't you tell us about changes in the oxygen uptake of the brain in aging?

Himwich If you determine brain metabolism in an adult human between 20 and 40 years of age, using the method of Kety and Schmidt you will find that it will be about 3 3 ml of oxygen per 100 gm. of brain tissue per minute (17)

Now if you go to an older age span, say from 40 to 60, and compare the average rates of brain metabolism, a slight decrease will be observed but it may be within the error of this method At a later age from 60 to 82, however, a significant fall is observed Freyhan, Woodford and Kety (18) found that patients with either cerebral arteriosclerosis or with senile dementia exhibited a brain metabolism which was 15 per cent lower on the average than that of young adults They made two suggestions They said it was possible that such a decrease was due to destruction of the brain, causing the abnormal mental symptoms. But they also said they could not draw a firm conclusion until they

studied other elderly individuals who did not exhibit pathological symptoms.

Then Fazekas, Alman and Bessman (19) did just that thing. They compared individuals of an advanced age who were apparently normal with patients who had signs of cerebrovascular disease or parenchymatous brain damage. Rather surprisingly they found that the reduction in cerebral metabolic rate was the same in both groups. Just recently Scheinberg *et al.* (20) extended these observations to a large number of aged individuals who were apparently normal, without any signs of clinical involvement, and they were able to confirm an average decrease in brain metabolism. Thus again no significant difference was found between the brain metabolism of an elderly person with symptoms of cerebral disorder and another equally elderly but symptom free individual. This means that for the aged individual we cannot have the same kind of a control that we have in the younger adult. In a younger person with cerebral destruction it is possible to disclose an impaired brain metabolism, for example, cerebral metabolic rate is lower in a patient with the irreversible damage produced by syphilis than in a healthy man of similar age. In the older individual this cannot be done. Apparently a decrease of brain metabolism is a sign of aging which occurs even in the absence of any clinical symptoms.

I would like to emphasize, however, that these observations depend on average values and just as there are some elderly individuals whose brain metabolism suffers a much greater decrease than the average fall of 15 per cent reported for later life, there are other individuals with values indistinguishable from young adults. In these persons the aging process has been retarded.

Shock: The difficulty is that in dealing with observations on an entire organ, like the brain, we cannot distinguish between effects due to loss of cellular elements and those due to diminished function of the cellular elements that still remain Certainly this is the case in estimates of kidney function, where our observations deal with net changes (21, 22).

Himwich: I think you put your finger on it.

Shock: Could you tell us something about age changes in oxidative mechanisms in tissue slices?

Himwich: May I answer both your remarks? The Kety-Schmidt method is an over-all one so that the results apply to the entire brain. It cannot pinpoint the lesion The conclusion drawn from the observation that the fall in brain metabolism occurs whether or not the elderly person exhibits brain symptoms would seem to indicate that it is not the severity of the lesion that counts but its localization If it is localized in certain strategic areas, neurological and psychiatric symptoms

of organic damage are found. If the lesion occurs in other parts of the brain the person remains symptom free

I would like to discuss briefly the chief enzymatic changes which occur throughout the life span beginning with birth and extending to the senium. For the present we must content ourselves to a large extent with studies on the brains of infrahuman animals.

The results on brain metabolism presented in Figure 34, were obtained on the mouse (23). The abscissae represent days of age, A standing for the adult. The ordinates indicate utilization of cubic mm. of oxygen per hundred mg of cerebral tissue per hour At birth, oxygen uptake is low and during the first few days it becomes even lower. Then there is a remarkable increase till a maximum is attained at about the end of the first month of life only to be followed by a slow and gradual decline in the adult.

The next series of observations, Figure 35, were made on rats varying in age from birth to 520 days (24). The last figure is roughly equiva-

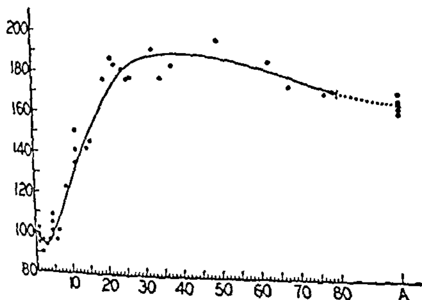


FIGURE 34 Changes in oxygen consumption of mouse brain from birth through maturity Age in days (abscissa) plotted against oxygen uptake, cu mm O_2 per 100 mg tissue per hr (ordinate) The oxygen consumption of the entire brain of the mouse is low at birth and may fall somewhat for the first two or three days thereafter Then brain metabolism rises rapidly to a maximum at one month of age after which there is a slight but significant decrease Reprinted, by permission, from Tyler, D B, and van Harreveld, A The respiration of the developing brain *Am J Physiol* 136, 600 (1942)

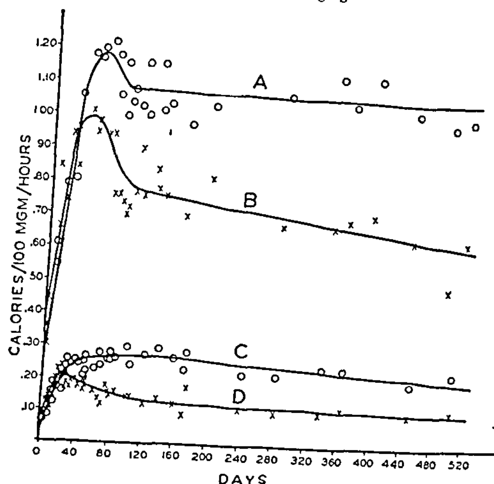


FIGURE 35 Comparison of the energy, in terms of calories, made available by oxidation (A and B) and by glycolysis (C and D) in the various stages of life in the rat The differences between A and C for the cerebral cortex and B and D for the brain stem reveal the inferior position of glycolysis for all parts of the life span These differences, termed the energy debt, show that in the adult, an energy debt accumulates more rapidly than in the newborn. This increasingly severe deprivation of energy with growth is the cause of the greater susceptibility of the adult to anaerobiosis Reprinted, by permission, from Chesler, A., and Himwich, H. E Comparative studies of the rates of oxidation and glycolysis in the cerebral cortex and brain stem of the rat *Am J Physiol* **141,** *513 (1944)*

lent to that of a man 45 years old Here again you see the same general trend, a rapid rise to an early maximum then a decline in early maturity followed by a leveling off of brain metabolism which remains approximately the same for some time thereafter. Curve A represents the oxygen uptake of the cerebral cortex and curve B of the brain stem. Reiner (25) confirmed the above results and observed a fairly constant

rate of brain metabolism up to two years, equivalent to a man aged 60. But he also studied another group of aged rats that survived longer than 2 years and found a definite falling off in brain metabolism. It is of some interest that the liver reveals a picture similar to that of the brain and its oxygen consumption remains approximately the same for two years When you compare the brain with the kidney you see that the brain is more fortunate because renal metabolic rate reveals a continual decrease with age (26)

Lansing: How about the peak that occurs at about 40 days of age?

Himwich: There is a rapid increase during early growth which

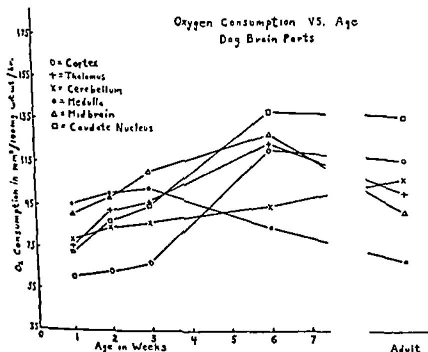


FIGURE 36 The values for the oxygen consumption of the various parts of the dog brain at 1, 2, 3, and 6 weeks and in adulthood are presented These observations reveal that the relative rates vary at different stages of development, for example, in the newborn the medulla and midbrain possess the fastest rates while the caudate nucleus and cortex have the slowest ones In the adult, on the other hand, the cortex and caudate nucleus consume oxygen most avidly while the midbrain and especially the medulla have the lowest oxygen consumption Reprinted, by permission, from Himwich, H E, and Fazekas, J F Comparative studies of the metabolism of the brain of infant and adult dogs *Am J Physiol* 132, 454 (1941)

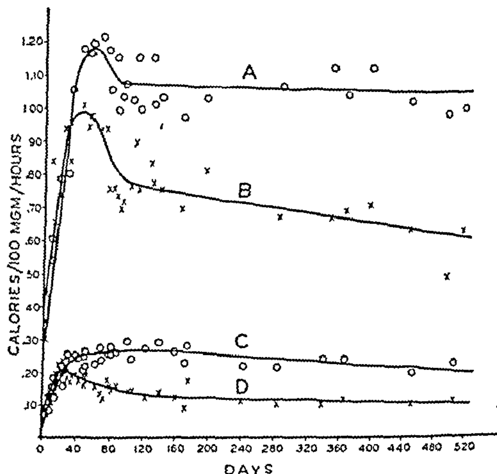


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Then all areas suffer a moderate decline till the animal becomes adult. Presumably they remain at these somewhat lower levels for a long time thereafter.

There is some hint as to the development of enzymes in Figure 37, (29). Again in the rat the observations reveal that cytochrome oxidase activity, an enzyme important in oxidations, is in its nadir at about the time of birth It comes to a peak at about the 33rd day and recedes somewhat in activity in the adult.

Where glucose is oxidized, as you know, it is split to pyruvic acid or lactic acid In the medulla lactic acid formation starts at a high rate and then comes down to progressively lower levels (30) The initial rate of the cortex is much lower but even in the young adult it is still exhibiting a slight increase We, therefore, have evidence that this anaerobic splitting of carbohydrate also follows a pattern similar to that of oxidations as can be seen by comparing Table IX with Figure 36 With glycolysis as with oxidations there is a reversal, the lower parts of the brain exhibiting a more rapid glycolysis earlier while the upper parts, though starting slowly, continue to increase for some time thereafter

TABLE IX

Lactic Acid Production of the Various Parts of Canine Brain Measured in Terms of CO_2 cu mm/100 mg. Tissue/Hour

Part of Brain	Age			
	Less Than 1 Week	3 - 6 Weeks	Approx 3 Months	Adults
Cortex	46	117	195	225
Caudate nucleus	73	157	256	229
Thalamus	104	160	243	193
Corpora quadrigemina	116	148	157	85
Medulla	144	118	61	30
Cord	87	38	18	20

100 cu mm CO_2 are released by the formation of 0 4 mg lactic acid which process in turn yields 0 128 calorie anaerobically The rates of lactic acid formation of medulla and cord progressively decrease after the first week while those of the caudate nucleus and thalamus are accelerated until the third month In the cerebral cortex the rate continues to rise somewhat even in the young adult

Reprinted, by permission, from Chesler, A., and Himwich, H E Glycolysis in the parts of the central nervous system of cats and dogs during growth *Am J Physiol* 142, 544 (1944)

ceases at early maturity and then cerebral metabolic rate remains relatively stable though exhibiting a slight decrease.

Lansing: Is that peak present in other tissues?

Shock: There is a similar peak in the resting metabolism of the human calculated on the basis of surface area at about two and a half years. Prior to this age, the metabolic rate rises—following this age, metabolism falls (27).

Himwich: Yes, it is similar to basal metabolic rate in the human subject. After rising sharply in the first 2 or 3 years of life BMR recedes till the age of 20 when it remains about the same till 40.

Apparently each part of the brain has its own history as seen in Figure 36, (28). If we start this discussion with the medulla oblongata, the part of the brain which is lowest anatomically and oldest phyletically we find that its metabolic peak comes at three weeks of age in the puppy. If, on the other hand, we consider a younger part of the brain, for example, the caudate nucleus or the cerebral cortex we observe that it attains its maximum at a later age, approximately at six weeks

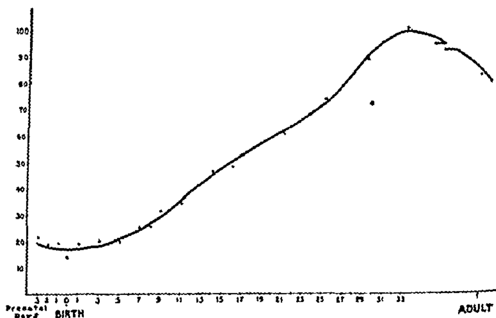


FIGURE 37 Cytochrome oxidase activity (Q_{ox}) in rat brain expressed as cu mm. O_2 per hr. per mg fresh tissue, remains at the same low value during the last days before birth and the first one postpartum and then rises rapidly to a maximum at approximately the end of the first month with a slight decrease in the adult. Reprinted, by permission, from Potter, V., Schneider, W. C., and Liebl, G. J.. Enzyme changes during growth and differentiation in the tissues of the newborn rat. *Cancer Research* 5, 21 (1945).

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Reprinted, by permission, from Chesler, A and Himwich, H E Glycolysis in the parts of the central nervous system of cats and dogs during growth *Am J Physiol* 142, 544 (1944)

Before the energy of oxidation can be used to support the function of any organ it must be stored in energy-rich phosphate bonds (31). Figure 38 presents additional data from the paper of Potter *et al.* (29) on ATP-ase, the enzyme that splits off energy-rich phosphate groups from adenosine triphosphate, the immediate source of energy for work. You observe how low its activity is at birth and how rapidly it rises during the first postnatal month.

Shock: What animal is that?

Himwich: These results were obtained in the rat.

Hisaw: Was that dehydrogenase?

Himwich: I have been stressing the changes of adenosine triphosphatase.

Hisaw: I thought you said it was dehydrogenase.

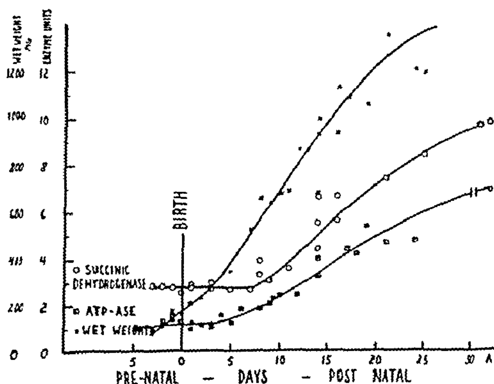


FIGURE 38 Changes in enzymatic activity in brain of young rats Succinic dehydrogenase activity (O) starts low and later during the first postnatal month rises rapidly to a value seven-fold that observed at birth Adenosine triphosphatase activity (□) is also slow for several days before and after birth and then the rate increases more than three-fold by the 30th day of life Reprinted, by permission, from Potter, V, Schneider, W C, and Liebl, G J Enzyme changes during growth and differentiation in the tissues of the newborn rat *Cancer Research* 5, 21 (1945)

Himwich: Yes, Figure 38 shows not only the adenosine triphosphatase but also the succinic dehydrogenase. The details of the latter are slightly different, for at birth its activity is somewhat higher than ATP-ase and this activity is maintained at the same rate for a few days but it too finally rises. In general the patterns of many enzymes are the same at this time of life. The presence of adenosine triphosphatase makes energy available and among other purposes it may subserve the formation of acetylcholine.

Figure 39 is taken from one of Nachmansohn's papers (32). The

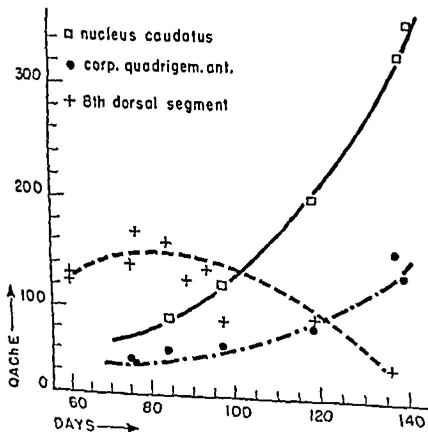


FIGURE 39 Changes of cholinesterase activity in brain and *spinal* cord of sheep embryos. Abscissae, days of gestation. Ordinates, *mg* of acetylcholine split per gram of tissue per hour. The caudate nucleus and the corpora quadrigemina ... gain in activity throughout ... tive, loses activity for the rem... from Nachmansohn, D. Ch... embryos. *J. Neurophysiol.* 3, 330 (1940) ... of sheep

observations were made on fetal lambs. Unlike the rat which is born blind and without righting reflexes, the lamb is able to see, stand and walk at birth. Physiologically the lamb is older and accordingly enzymatic changes which occur postnatally in a rat are observed in the lamb while still *in utero*.

The cholinesterase activity of the 8th dorsal segment of the cord begins to lose its activity during gestation at a time when the caudate nucleus continues to exhibit a rise.

Finally, Figure 40 presents a picture of the cholinesterase activities in the brain of the growing rat (33). In this case the maximum for the entire brain is observed at about the 25th day of age and a much lower activity is noted in the adult.

One of the gaps in our knowledge, and a serious one, is the fact that we do not have adequate data for brain enzymes in old age. We do not know the values of oxygen consumption or whether they decrease in either humans or animals. We have determined that the rates of glycolysis are impaired. But there are many other types of enzymes that await study for the late extreme of life, for example, two enzymes mentioned above, adenosine triphosphatase and cholinesterase.

Dr Lansing would rather not go beyond observed facts and I agree with him but it is permissible to suggest that in the aged many enzyme systems will decrease in activity Such changes are in accordance with

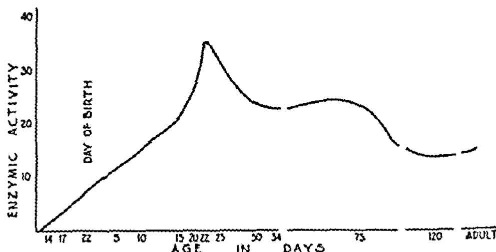


FIGURE 40 Cholinesterase activity in the brain of the developing rat expressed in terms of mg of acetylcholine split per gram of tissue per hour Cholinesterase activity is much greater in the brain of a rat three weeks of age than in that of an adult Reprinted, by permission, from Metzler, C. J., and Humm, D. G. Determination of cholinesterase activity in whole brains of developing rats *Science* 113, 382 (1951).

the decrease in basal metabolic rate observed in the human during the senium

Carlson: May I ask a question and make a suggestion? Now, the aging of the white cells of the blood of man and animals is something that goes on right along no matter what changes in the total age of the individual, or whatever changes there are in the blood. Now, if we could for a moment concentrate on possible methods of studying the aging in that group of cells, that might give us some clues as to aging *per se*, rather than stress or accidents, or what-have-you, because that would tend to remove all those other uncertainties

Gross: I just wondered if I might possibly bring up a point on this particular issue as a follow-up to something which Dr. Chow said. He makes a strong point for the importance of the environment of the organism, and I should like to extrapolate and say that in considering cell physiology, the environment of the cell may be an important factor here as well

One of the characteristics of an animal tissue is its function *in toto*. All parts are interdependent, and one portion of the tissue which is very frequently neglected is the milieu in which the cells are formed and embedded, the extracellular substances From a simple but general histological relationship I think one could speculate as to one possible function of this milieu of the cells

The general scheme of almost any tissue where there are cells and extracellular substance is roughly this between cells and blood or lymph vessels there is always an intervening layer of connective tissue. This gelled matrix varies greatly in thickness and composition from tissue to tissue There is no free space for water and salts to trickle around in The components of the connective tissue are chemically reactive and capable of forming selective diffusion barriers.

Whatever metabolites travel between the blood and the cells and whatever breakdown products come from these cells to the blood, pass through this "filter bed," and it is possible—in fact, there is some evidence I will mention in a moment—that this tissue can let certain materials pass through selectively, bar others or diminish the speed of movement of *certain* other substances and salt and water

In the aging process there is very definite change in this milieu, at least in certain tissues Although structures like liver and kidney and other parenchymal organs, haven't been studied yet, we know that the connective tissue of skin, for example, changes in composition. In the young, it is highly gelled, and solvated, there is a large amount of this amorphous matrix As the animal ages, the gelled amorphous ground substance is slowly absorbed one way or another, and fibrous collagen, more inert material fills in the space In the aged animal a fresh frozen

section of the skin will readily disperse into collagen fibrils whereas in the young animal, the voluminous gelled ground substance must first be treated with an enzyme to free the collagen from the matrix.

Is it not possible that the changes which take place in the connective tissue with aging may modify the diffusion of metabolites, electrolytes, water, to and from the cells? Perhaps even storage of the pigment granules, might be a result of altered extracellular permeability. The whole concept, however, remains to be proven.

There is fragmentary evidence from several directions suggesting a significant role of connective tissue in fluid transport. Cogan *et al.* (34, 35) have studied the selective directional diffusion of dyes, water and salts through the cornea. Friedenwald and Steihler (36) and Kinsey (37) have found that the stroma of the ciliary body plays an important role in the secretion of the aqueous humor; similarly the stroma of the choroid plexus is involved in the secretion of the cerebrospinal fluid (38).

Ussing (39) has extensively studied fluid transport in frog skin and here also the connective tissue is functional. Sylvén's group in Sweden (40) has been investigating the diffusion of various substances through cartilage and find interesting examples of selectivity between sugars. There is an extensive literature on the reactivity of connective tissue to hormones, reactivity which involves changes in hydration and electrolyte content (41, 42).

Cowdry: The cells that are being fed depend for supplies and elimination of waste not only on the character of this barrier, or filter bed, between them and the blood vessels, but also on many other properties of each tissue Among these are to be included the volume per minute flow of blood per cu. mm of tissue, the permeability of the walls of the blood vessels, the abundance, scarcity or absence of lymphatic capillaries draining fluid from this filter bed, and the presence, or absence, of membranes, or partitions, in the filter bed that restrict or direct the flow of fluid in it Of course, the density and the character of the cellular population will also influence the character of the tissue fluid in which citizen cells are residents and the climate, depending partly on locally established and regulated differences in temperature, is not to be ignored In brief, the factors that determine the conditions of cell life are many and difficult to measure quantitatively There is homeostasis, or maintenance of like states, in the blood and heterostasis, or maintenance of different states, in the several tissues of the body, each adjusted to the special functional needs of its cellular inhabitants.

Gross: The only point I wanted to make was that there are anatomical relationship between cells, connective tissue stroma and blood and lymph vessels which could permit the stroma to mediate the flow

of vital materials between the blood and the cells. So that whatever goes to the cells or comes from the cells just doesn't trickle; it goes through some sort of filter bed

Cowdry. This matrix would become very small indeed in the stratified epithelium, wouldn't it?

Gross: Yes, Dr. Cowdry, but even very thin membranes can exhibit remarkable selective permeability.

Cowdry: I admit, of course, that all cells that are alive must be in contact with fluid, more or less, and that there is, therefore, between every cell some fluid substance to filter through which is your reactive membrane, isn't it—that fluid?

Gross: But probably not bathed in any significant volumes of free fluid although there are no estimates of the consistency of extracellular gel matrices

Cowdry: There must be movement

Gross: Yes, surely there is.

Wislocki: Until very recently, the material between the capillaries and lymphatics and adjacent epithelial cells was regarded as a network of collageneous and elastic fibers with spaces between them containing some kind of physiological salt solution

Sylvia Bensley was the first one, I believe, to draw attention to the fact that the connective tissue spaces are occupied by gels of protein nature rather than by an aqueous fluid containing merely salts and metabolites In the intervening period, between about 1933 and the present year, it has come to be realized, as Dr Gross has pointed out, that protein gels form a predominant and functionally important component occupying the spaces between the cells and fibers of connective tissues Furthermore, beneath epithelial layers and intervening between them and capillaries, these gels form continuous sheets called basement membranes These are denser and more readily stainable than the gelatinous components of the more loosely arranged portions of the connective tissues These basement membranes are believed to play significant roles in regulating the transfer of all kinds of metabolites from capillaries to epithelial cells and vice versa

Shock Some studies have been made on age changes in enzyme activity of articular cartilage Rosenthal and his co-workers have shown a decline with age in the respiratory power of articular cartilage taken from cattle over the age range of from six months to 11 years They measured the Q_{O_2} of tissues with and without the addition of methylene blue as well as the rate of glucose dehydrogenation and anaerobic glucolysis The ratio of glucose dehydrogenation to glucolysis did not change very much with age From this they concluded that the dehydrogenatic activity of the cartilage cell decreased very little with advanc-

ing age. They ascribed the age decline of total respiratory activity to a failure of the oxygen activating component of the system (43, 44, 45)

Hoskins: Is there any other discussion of this important principle of enzymatic adaptation to circumstances? What is the common belief among biochemists on the constancy of the enzyme patterns or their adaptive changes as circumstances change?

Himwich: In lower organisms there are many observations indicating adaptive changes of enzymes. The phenomenon of the sulpha-fast bacteria furnishes an example The idea appears logical. I believe that Dr. Chow's data afford suggestive evidence in favor of that point in mammals.

Shock: I think the observations mentioned by Dr. Carlson may also indicate the adaptation of metabolic processes to changed environmental circumstances. As I recall the observations, animals that were placed on reduced caloric intakes for a period of time, and then returned to a diet that had previously just maintained their body weight, gained weight on this same diet The caloric intake, required to maintain body weight, was lower after a period of starvation than it had been before Isn't this an indication of some change in the effectiveness of ultilization, perhaps at a cellular level?

Himwich: I think Dr Chow has an answer for that; for example he told me today that some of the material usually eaten may be wasted under one kind of environmental condition but may be utilized more efficiently in another environment

Shock: What do you mean by "wasted?"

Himwich. I would like Dr Chow to discuss his point

Chow: We had some experiments on the effect of another type of stress on nitrogen metabolism We took two groups of animals. One group was put in a soundproof room, and the other group was teased with noise They were fed a diet which would be sufficient to maintain their body weight These two groups were given the same amount of diet. One group was subjected to stress by tapping the cages, making noises, and suddenly flashing light on them, so that every couple of minutes these rats would jump These animals did not utilize the protein We found a considerable amount of protein in the feces In other words, the digestion of the protein had been altered.

Shock: In the human, the amount of nitrogen excreted in the stools is strikingly constant (1-2 gm N per 24 hours) in spite of wide fluctuations in the daily nitrogen intake (from 3 5 gm to 28 gm N per 24 hours) (46) We have given elderly males daily nitrogen intakes ranging from 4 gm to 18 gm per day with no appreciable change in fecal N excretion. These subjects absorb the nitrogen and excrete the excess by way of the urine. Thus, I think we must agree that the extra

nitrogen administered gets into the metabolic pool, even though it may not be retained to any great extent.

Coudry: I was interested in what Dr. Chow just said in regard to mice, because when you keep one mouse per cage as compared with several mice per cage, the frequency of breast cancer is distinctly different in the two groups. It was less in the case of the crowded mice which are more active and perhaps get less food.

Frank: Roy Hoskins' questions raise another point. I have been very much concerned for years with some understanding of the situation that has been studied so often. People react in the classic fashion to an emotion-producing situation, as Cannon and others have described (47). But then, we see not only Selye's alarm reaction (48), but we also see people who go around continually showing anxiety How are we to understand a person who is continually subject to chronic stress, who suffers from this chronic anxiety? Can we say that somehow or other their basic physiological and metabolic processes have been permanently altered so that those people are always alert, ready to react as if threatened? I offer that as at least one situation in which environmental stresses may give rise to a shift or a change that becomes more or less permanently organized

We are seeing a functional or physiological analogue to what you would call a structural lesion It is a shift from the so-called normal structure to an abnormal situation involving a waste of energy, if you please, because a person is always ready to respond even though nothing happens This is an area that we ought to study since anxiety, or stress, or guilt, seems to be a very important component in the aging of an individual

Hoskins: One other point of take-off is in the change in pediatric practice over many years Babies in institutions who have a very placid, rigid way of life do not flourish at all The physiologic mechanisms become abnormal, as shown in the state of growth, state of health, and so on There must be mechanisms to implement such happenings I am rather fascinated by Dr Chow's suggestion that these environmental circumstances may change the enzyme picture, and that possibility, as you say, should be followed further

Hisau: Isn't this, though, the point what we might be overlooking is the remarkable adaptability of the animal, that is, an animal put in a strange environment is capable of adapting itself to that particular environment. It was shown back in the 1880's by Semper in his book on the relationship of animals to the environment (49) He describes the reactions of many species from protozoa to mammals, to a great variety of conditions showing that animals in general have a remarkable ability to become adapted or acclimated to radical changes in their

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We are seeing a functional or physiological analogue to what you would call a structural lesion It is a shift from the so-called normal structure to an abnormal situation involving a waste of energy, if you please, because a person is always ready to respond even though nothing happens This is an area that we ought to study since anxiety, or stress, or guilt, seems to be a very important component in the aging of an individual

Hoskins· One other point of take-off is in the change in pediatric practice over many years Babies in institutions who have a very placid, rigid way of life do not flourish at all The physiologic mechanisms become abnormal, as shown in the state of growth, state of health, and so on There must be mechanisms to implement such happenings I am rather fascinated by Dr Chow's suggestion that these environmental circumstances may change the enzyme picture, and that possibility, as you say, should be followed further

Hsiau Isn't this, though, the point what we might be overlooking is the remarkable adaptability of the animal; that is, an animal put in a strange environment is capable of adapting itself to that particular environment It was shown back in the 1880's by Semper in his book on the relationship of animals to the environment (49) He describes the reactions of many species from protozoa to mammals, to a great variety of conditions showing that animals in general have a remarkable ability to become adapted or acclimated to radical changes in their

physical organic environment. However, the time required for such adaptations varies with the situation and sudden changes may be very distressing.

Chow's remarks regarding the influence of noise reminds me of an incident that occurred last year in our animal colony. We have a large breeding colony of rats for which careful records are kept as to weights, breeding performance, age, etc. and the number of young per litter is limited to five.

Last year some of our colleagues in another department persuaded us to let them keep a group of dogs in a room a short distance from our rat colony. The dogs barked and carried on, as dogs do in captivity, and the average weight of our rats at 22 days of age declined remarkably. When they took the dogs away, our rats returned to normal weight. The loss in weight seemed to be due to the disturbance of having the dogs in the vicinity.

Bourliere: I have been much interested by what Dr Chow said about the importance of various environmental stresses on the speeding up of the processes of aging of his white rats. Ecologists have recently emphasized the role played by "stressing" environmental conditions on the dynamics of wild populations of rodents.

It is now well known that natural populations of some species of mammals and birds may show quite regular cyclic fluctuations in numbers In the old world voles, mice and lemmings are usually very abundant during one or two years, and quite scarce the next few years. In North America the same phenomenon has been observed in various Microtinae and in the snowshoe hares. In both hemispheres these "vole outbreaks," which show a quite definite periodicity (every 3-5 years for the smaller species, every 9-11 years for the larger ones), are followed by a strong periodic increase of the numbers of carnivorous species (various species of foxes, Canadian lynxes, snowy owls, etc) which feed upon these small rodents A more comprehensive review of these population cycles may be found in my recent book on the comparative ecology of mammals (50)

The important point for our present discussion is the mechanism of these cyclic fluctuations in numbers and especially of the very quick and impressive population decrease which follows every "peak" in population density. For many years, ecologists thought that the cause of such a dramatic mass mortality was due to the spread of some bacterial or virus disease among the population But a number of very many careful investigations showed that such an explanation was definitely wrong. Furthermore the work of Green and Evans (51, 52, 53) on the snowshoe hare pointed out that the mass mortality which was responsible for the rapid decline of their population was probably due

to some "shock disease" whose symptoms were convulsions, progressive torpor, hypoglycemia, increase of the non-proteic nitrogen of the blood, etc Recently Christian (54) published a very stimulating paper on that problem, in which he suggests that the various environmental stresses due to the overpopulation of the environment are probably responsible for the rapid decline in numbers of the population. On the another hand Errington (55) has shown that the overpopulation in itself is responsible for the decline of the fecundity in the muskrat. Whichever may be the results of the investigations now undertaken in various laboratories, the importance of various environmental stresses, i. e. of environmental factors which are outside the normal range of adaptability of the species, on the population dynamics in natural conditions, appears to be greater than previously suspected.

Hoskins: Isn't there also a change in the proportion of the sexes at birth in a strenuous environment as compared with a more adequate environment?

Bourliere: I never heard of any modification of the sex-ratio occurring during the cyclic fluctuations in numbers of the populations of small mammals It has only been shown that the number of young per brood and the number of broods per year varied widely during the population cycle A lot of work is now being done on such problems, especially by the Bureau of Animal Population in Oxford University, and it appears that population cycles are probably more numerous than commonly realized, especially in arctic, sub-arctic and desert countries.

Hisaw: A number of species of birds and mammals have been studied by our conservation people, who have observed similar wide fluctuations in population density

Bourliere: Yes, there is important American literature on this.

Hoskins: I have a question I wanted to ask Dr Gross in relation to his work on the filter bed of the cornea and other tissues Was anything done in that connection with hyaluronidase, or is the hyaluronidase content of these filter beds known? Is that a possible factor in the selective permeability?

Gross: I do not know that anybody has as yet isolated a real hyaluronidase from connective tissue I think hyaluronidase has been found in various tissues, such as the testes, leech heads, venom of various sorts, and bacteria, but no one has demonstrated to everybody's satisfaction that there is a hyaluronidase in connective tissue

Shock: I think Dr Gross has emphasized the concept that the physiological behavior of a tissue is dependent not only on its enzyme activities, but also on the nature of the surrounding environment. Hence, I would like to return to the question of whether the use of tissue cultures for the study of cellular changes with age does not offer great potentialities.

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(58), or more, of adult serum solution, will give you poor cell growth and poor cells, cells that won't last long. We are not able at this time to say what factors are present which bring about these changes. Not all adult sera behave this way. A few perhaps could be shown to maintain cells indefinitely. We have some evidence for this when dilute adult sera are used. Especially, in the case of the younger diluted sera, from the fetus or from young adults, we find that some cells will proliferate quite well for a long period of time, in fact, indefinitely

We have here an important facet which deals directly with the humoral aspects of aging, one which can be carried on in many directions The influence of diets of various sorts can be studied The hosts can be bled under all sorts of physiological conditions, and the humors obtained from them can be assayed on standard strains of cells, and thereby give us leads on what tends to accumulate or change in the extracellular fluids of the aged individual

We have conducted a few experiments of this sort which show that if one dilutes the old serum, one tends to nullify the effects that one sees from the higher concentration of the old serum

There is one other facet here that I should mention It pertains to our inability to provide media substrates of biological fluids for cells that are free of viruses Dr Bang and I have found that the tissues of fowls (59), for example, can give us nonspecific degeneration *in* tissue cultures grown *in* homologous media Fortunately, under the impact of our curiosity, we have made some electronmicrographs of some of these degenerating cells, and we have found that some of these cells were being killed by a presumed contaminating virus, thus indicating that the source material for our tissue culture media and cells were probably obtained from virus carrying hosts Here, one of a whole group of chronically carried viral agents that one finds in chickens could prevent us from doing a clean job Virus-free stocks for source material would help to evaluate age changes uncomplicated by such infections

There are many facets on which one might work in connection with the study of the problem of aging, and I do not think that today we (those working with living cells) are prepared to say that we know of very many important differences between an old and a young cell One might begin by looking for genetic losses in a rundown colony of paramecium, or some other single cell form One might expect to have losses of such character that the surviving population could never regain those genetic components that had been lost to the population as a whole during the so-called aging process

In mammalian cell populations, I think the day may come when we can talk about losses in a genetic sense Yet, I feel that in the presence

Gey: If that question is directed to me, I say this: that we don't have enough information as yet to make any very strong statements We can go back quite a long way to the work of Dr. Carrel and Dr. Fischer, and others, who worked in the early days with the so-called continuous or strain cultures, and read some statements of theirs to the effect that when lines or strains of cells are established from young and old tissues, they seem to manifest pretty much the same proliferative capacity (56). This, of course, would mean that they are studied under similar circumstances.

There may be some differences, let us say, in proliferative capacity with aging. I can cite the simple experiment which I reported at the Second International Gerotological Congress in St Louis, of some work that I did with Dr Joseph McManus We wanted to get some idea of the proliferative capacity of the epithelial cells at the basal layer and from there on up to the cornified layer By making multiple lesions with a laminated instrument, containing razor blades, we produced a lot of microscopic sharp cuts, some of which went just beneath the epidermis and others just part way through Then by taking these bits of wounded tissue from young and old individuals and subjecting them to the stimulating circumstances of tissue culture, we found that dividing cells were found in the gap that was produced in the lesions in both the young and the old tissues, even as early as the first day Serial sections were required to evaluate the response Dividing cells were found at the basal and the spinous layer We were really trying to get a lead on the low proliferative capacity of basal cell cancer

This, of course, is only one circumstance It refers only to one tissue system It is quite possible that if research were limited to, let us say, the study of the cornea, we might find that the cornea shows no marked signs of aging It may well be that the more favorable circumstances found in the cells of the cornea, from the endothelium right out through Descemet's membrane and out to the epithelium, are due to the fact that this tissue is more or less remote from the cumulative circulatory stresses to which other tissue systems may be subjected

The best that we can do, it seems to me, is to categorize our tissue systems and say, "Well, this is the system we should work with because we find in this tissue system the best evidence of aging." Dr Simms has done work of that sort and can speak for himself.

The other way, I think, that one could go about it is to start with the humors of the young and the old individual (57), and here we have a few experiments carried out about 1935 that I think are important If one takes a standard strain of adult human fibroblasts, puts them into a dilute serum solution, with a balanced salt solution as diluent, one finds that a rather high concentration, of the order of 80 per cent

(58), or more, of adult serum solution, will give you poor cell growth and poor cells, cells that won't last long. We are not able at this time to say what factors are present which bring about these changes Not all adult sera behave this way A few perhaps could be shown to maintain cells indefinitely. We have some evidence for this when dilute adult sera are used. Especially, in the case of the younger diluted sera, from the fetus or from young adults, we find that some cells will proliferate quite well for a long period of time, in fact, indefinitely.

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of abundance of nutrients, the situation one often finds in nature, this may never occur; whereas, under the conditions of experiments like those of Dr. Jennings, for example, where they were using chemically defined substances, they did find that the system ran down. But we are talking about situations where we can assume that there is an abundance of nutrients, and, even here, we still see conditions of aging, whatever they may be

Of course, this is a very difficult thing to define. What is aging? I have never heard a good definition, and I do not think that I shall, especially, if one must limit his statement to a particular cell system.

There are many bits of evidence that cell strains can remain stable for a long period of time In our laboratory, we have several lines of cells that Mrs. Gey has cultivated from 15 to over 20 years I can give you an example of a good one. I must go out of the realm of normal tissues now and into a consideration of what happens to a cultured sarcoma of the rat Walker Sarcoma No 319 of the rat, grown in a totally heterogeneous medium, containing no rat protein constituents. will remain a rat sarcoma (still producing tumors in rats) even after 20 years of growth in an heterologous substrate Our impression is that the rat cells take what they wish and pay no attention to the rest In terms of microscopic studies that have been made on the distribution of materials in a living cell's metabolic pool, it would seem that a great many things go in and come out continually. So it is quite possible that the rat cell does take in a lot of these things by pinocytosis but uses, let us say, intracellularly, only those things which are essential to its function This heterologous situation, rat cells and strange media, demonstrates the stability of this rat sarcoma cell

There are other examples of stable lines of cells, and we have a great many of them in the laboratory Some are normal cell strains.

Then, too, we have come across variations which carry us over into malignancy. We have been fortunate to be the first ones to observe a so-called "spontaneous" conversion of normal cells into malignant cells in continuous tissue cultures. We are not able to account for the specific circumstances under which this set of transformations occurred In the laboratories of Dr. Wilton Earle and of Dr Katherine Sanford, of the National Cancer Institute, they have been able to get a number of tumor conversions from cell systems (mouse cultures) by inoculating homozygous hosts.

In doing any of this work, of course, one must be careful to evaluate that with which he is dealing, because in these highly inbred mice, one can easily transfer or transplant tissues from one animal to another without getting much in the way of an adverse reaction toward them A jump to malignancy is something that may not be as clearly under-

stood under these circumstances as under the conditions of a more heterozygous system, as occurs in man.

I have always been interested in an aspect of aging that Dr. Engel asked me to comment on earlier during this conference. It is the question of what constitutes the whole system of factors that obtain in the individual which keeps us in a state of dormancy. A tissue which is mechanically severely injured, uncomplicated by microbic flora, undergoes a very dramatic series of cell multiplications, with great increase in masses of cells, and then after a time this whole thing settles down, and with it, there is a loss of redundant cells, progressively, and finally a fairly good reconstitution of the tissue.

Under the conditions of malignant transformation, however, one sees entering into the picture a new kind of cell which doesn't appear to [illegible] situation in the host of origin. Yet, [illegible], and put it into a [illegible]nd of stroma, one does see evidence of differentiation. I could show you a very beautiful example of a cultured strain of human epidermoid carcinoma cells (Strain He La) that we have established over the past few years, which, when put into the guinea pig's eye, will go ahead and grow rapidly on the foreign underlying stroma and progressively destroy foreign tissues Where these undifferentiated cells come into apposition with the newly created basement membranes, they line up beautifully and here appear to respond to the formative stimuli that are present in the foreign tissues, indicating that they haven't really lost their ability to respond to these organizing factors In the host of origin, this tumor was quite undifferentiated

In this area, therefore, I feel that a great deal can be learned about what goes on in certain tissue systems, especially where dormancy factors or growth restraining factors are operating And I think it is important to stick to certain tissue systems if you have the belief that in this tissue you can learn something about the aging process. It all depends upon your concept of what is aging in a selected tissue

Frank: We have for a long time been speaking of target organs. I have questioned this idea of a target organ which assumes that the pituitary, for instance, can shoot something to a specific organ Actually the pituitary secretion is poured into the blood stream and each organ selects and absorbs what it can use Now, that makes it more dynamic and it makes it necessary for us to develop something which might be called a "field theory" to handle this environmental situation (60).

As Warren Walter pointed out (61), for generations we have been studying "disorganized complexities," and we have made enormous strides by fractionating them into variables and finding the relation

between two variables. Today the exigent problems in science call for the study of "organized complexities" in organisms and society, which cannot be handled in the old way. Many of the familiar concepts and methods that we have carried over from the study of "disorganized complexity" are no longer adequate for the study of "organized complexity" through time. We must emphasize that aging is a problem of organized complexity and realize that we cannot go on treating the aging organism as a disorganized complexity.

I am not objecting to doing all the fractionations that may be useful but we must bring the discrete finding back into the organized field. This is a biological necessity

I wonder whether the concept that Hoskins worked out in studying schizophrenics might not be fruitful in the study of aging—the individual becomes "physiologically clumsy" in later years and cannot maintain his organism as before, but the clumsiness is not specific—it is a way of functioning

Simms. I should like to make some further comments in regard to the question of applying tissue culture techniques to the study of aging. Dr. Gey has just indicated that in his experiments he found that older human-adult serum was less stimulating to growth than younger adult serum

Gey. And produced very profound degenerative cytological changes

Simms: In our laboratory we got just the opposite results, working with a different type of culture Using adult chicken aorta fibroblasts for the source of our cells, and making observations on chicken plasma from chickens of different ages we found that the older serum, or older plasma, was more stimulating to growth than the younger, up to about three or four years of age of the chicken, and from thereon it remained more or less constant We had animals up to about nine years of age, and the plasma from those animals was more stimulating to the growth of the adult chicken aorta fibroblast than the serum from young adult chickens.

Gey. They are in primary explants, I take it, and without continuous cultivation.

Simms· Yes

Gey: I am talking about maintenance through continuous cultivation, for a period of at least four months for the experiments on adult human fibroblasts in young and old sera (human)

Simms: In regard to the tissue, we found that the tissue from the older animals grew with a shorter lag period and with a faster growth rate than did that from the younger animals So that under the conditions of our experiments we reached a different conclusion from what you did under the conditions of your experiments

Hoskins: I can't hazard any guess as to the teleology there. You are dealing with a fibrous tissue, and aged people go in extensively for fibrous tissue.

Simms: It is dealing with a fibrous tissue, and from the adult, and we were observing, as Dr Gey just commented, the initial growth from the original explant taken from dormant tissue in a living animal

Another comment I should like to make is that in tissue culture work we are limited in the number of things that we can observe or measure The easiest thing to measure is rate of growth of the tissue, but in the adult animal growth is not an important process

Shock: Why couldn't you grow cells in tissue culture and then subject them to the cellular fractionation techniques for enzyme and biochemical studies? Is this a technical impossibility?

Simms: No It may not be impossible—but it would be difficult In plasma clot cultures the cells constitute only about one per cent of the volume

Shock: What I am trying to get at is this fundamentally can't you observe everything in a cell obtained from tissue culture by the same techniques that have been applied to tissue slices?

Gey: You can do any of the things that you do with any other cell system In fact, I would say that the slice system is the poorer method It gives you a bit of an idea of the *status quo* of that tissue plus the factor of injury in getting out your specimen Then, too, you have another aspect which is most important, and that is the ratio between acellular and cellular components in the tissue mass with which you are dealing This, of course, varies considerably with age

Shock: But isn't a tissue culture more free of extraneous non-cellular material than a tissue slice?

Gey: We can prepare cellular preparations with are spherical masses of perfectly naked cells without any other material present except the medium

Andrew: I just want to say that although we have seen many differences between cells of young and old individuals, that is, in a general way, you can't say the nucleus looks so-and-so *in an old cell*, or the cytoplasm has a particular type of mitochondria, unless you limit yourself to the discussion of certain cell types. You can say that a Purkinje cell is very likely to show such and such changes in an old animal as compared to a young animal Of course, you can't use the same criteria then in speaking about a cell of the salivary glands And I don't believe that you would expect to, because there are so many diverse cell types in the body at any age, and we have to study the cells as individual groups

Somewhat the same thing is true in regard to the question of the

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morphological characteristics of malignancy, as I understand them. At George Washington University we invited Dr. Ross MacCardle from the National Cancer Institute to come down and give an hour lecture with the title "The Characteristics of the Malignant Cell" to the freshman students. It was a very enjoyable lecture, but the students were somewhat disappointed when the conclusion was that there are no characteristics as such of a malignant cell, no one or two or three characteristics to which one can point.

Well, the same thing is true in a way of the senile cell, and yet it does not detract from the importance of studying the differences that we see in particular groups of cells from young and old individuals. Within those particular groups the differences often are very definite.

One thing that might be stressed with regard to differences in cell population, in young and old animals, is the greater degree of heterogeneity which comes with increasing age The cells of a given tissue in the young animal often seem to be very much alike, sometimes like a group of babies to the unpracticed eye. At least, they look very similar, but frequently in the senile tissue there is a considerable variation of the cells, even neighboring cells, variation in the character of the nuclei and the number of mitochondria, and the appearance of the Golgi apparatus. That sometimes is so great that we have to speak of the altered cells by a special name, such as the onocytes in the salivary glands and also in some other organs. So one general type of change may be said to be this increasing degree of heterogeneity in cell populations in senile tissues.

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Determinate growth is exemplified by the higher vertebrates, mammals and birds In these warm-blooded organisms the life cycle may be roughly subdivided into three consecutive phases. growth, maturity, and senility. During the growth period the organism as a whole, as well as its various organs, increases in weight and in size until a specific adult size is reached. Some environmental conditions may slow down or speed up the growth rate to a certain extent, but these ecological factors do not alter very much the morphology of the mature animal. In this respect, genetic factors are usually more important in the warm-blooded vertebrates than ecological factors During maturity the size of the animal remains fairly constant even if its weight increases slowly due to fat deposition. The peak of fertility of such mature homeothermic animals is usually reached early after the time of sexual maturation and decreases more or less rapidly afterwards

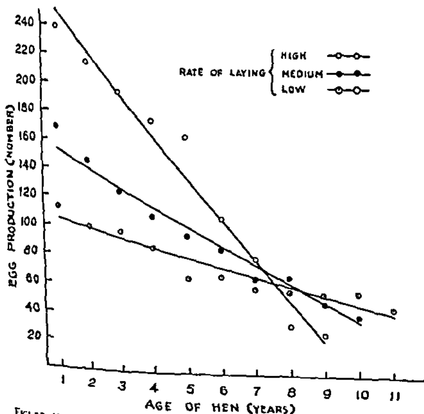


FIGURE 41 Decrease of egg-production with age in the fowl. Reprinted, by permission, from Romanoff, A L., and Romanoff, A J *The Avian Egg* New York, Wiley, 1949

THE ROLE OF COMPARATIVE PHYSIOLOGY IN STUDIES OF AGING

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I WANT TO present to you some data, some personal and some reviewed, on the comparative physiology of aging. I don't intend today to try to review the whole field of the comparative physiology of growth and aging. But I should like to emphasize some of the aspects of the comparative physiology of growth and aging in vertebrates.

The physiology of aging has been studied chiefly in a very few laboratory mammals—mainly, rats, mice, and guinea pigs—and also in healthy and in more or less disabled men. But aging is a very fundamental characteristic of living matter, and the study of this phenomenon in the lower levels of animal organization would probably give us a better understanding of old age in higher vertebrates and man.

Everybody knows the pioneer studies of Pearl and his associates (1) on drosophila and other invertebrates. MacArthur and Baillie (2) were the first to confirm experimentally Rubner's (3) theoretical conclusion that the life span of an organism is condensed or lengthened as the average metabolic level is raised or lowered. On the other hand, the experiments made by Lansing (4) on rotifers shed a new light on the importance of cytoplasmic factors in aging Such experiments on the lowest forms of animal life are of the utmost importance from the standpoint of general physiology, and they may help us to understand more complex phenomena occurring in higher vertebrates. But the general plan of organization is so different in protozoa, invertebrates, and vertebrates that one may ask if it is fair to extend to mammals and men some conclusions drawn from experiments made on paramecium, drosophila, cyclops, or cantaloupe seeds No doubt the basic vital phenomena are the same in the various phyla, but the role of integrative organs is so great in dynamic phenomena, such as growth and aging, that it would be better to compare living organisms not too different from each other.

As a matter of fact, growth and aging patterns are almost as different in the various classes of vertebrates as they are among the invertebrates themselves. It is now well known that in both groups there are two different growth patterns, at least—determinate and indeterminate, and that aging characteristics vary accordingly.

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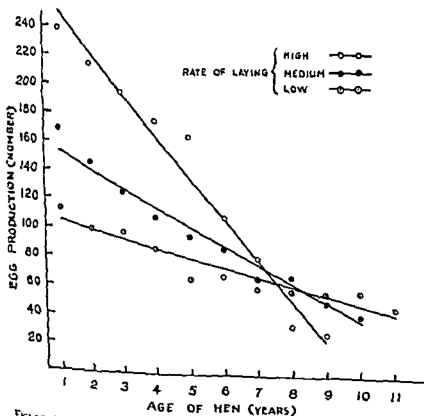


FIGURE 41 Decrease of egg-production with age in the fowl. Reprinted, by permission, from Romanoff, A L, and Romanoff, A J *The Avian Egg*. New York, Wiley, 1949.

Figure 41 is a graph from the recent book of the Romanoffs (5), showing the variation with age of egg production in fowl. You will see that in various kinds of fowl there is a regular decrease in egg production from the first year of age up to the eleventh.

These graphs are especially valuable because they are based on a very large number of observations. An objection could nevertheless be that fowl are domestic animals, therefore that their egg production is probably very different from that of wild birds This is true, but what we know at the present time of the variations of fertility in wild species agree pretty well with this fowl pattern However in some penguins the egg production of the younger females has been shown to be less during their first breeding season than during the next ones, declining again in the older females.

Carlson: Does this apply to birds that lay eggs and hatch them, say, once a year?

Bourliere: The few data we have on the age variations of egg production in wild birds seem to indicate a decline in fertility in older females.

Chow: Are the eggs laid identical?

Bourliere: Yes, as far as we can now be sure of that, the eggs laid are morphologically the same.

Lansing: Have any studies been done on the characteristics of eggs laid at different points in the life span of the hen?

Bourliere: I don't know.

Lansing: I recall the work of Jennings (6), not on chickens or birds, but on rotifers. In that work he found elaborate morphological and physiologic changes from day to day in the characteristics of the eggs laid at different points in the life span

Bourliere: As far as birds are concerned, I don't think there are very great age changes in egg morphology It is well known that some types of coloration and some color patterns of the egg shell may remain constant during the whole life span of the same female But it is quite possible that the age of the mother has some influence on the hatching success of the eggs

Engle: I wonder whether that is true in the domestic fowl. The domestic fowl has been bred for egg production, since it is an egg-laying machine, and there have been scores of studies in the agricultural colleges in this country that show a life-long production of eggs But everybody knows that when the pullet starts to lay at age 6 months, it lays a small egg and will do so for a period of about six weeks. Then the egg reaches its maximum size, the size which is acceptable to the market. Then that egg size is maintained, and the only change in the curve is decreasing production The immature fowl produces eggs that

have a low fertility potential, but they are also small eggs, and there are more deformed eggs in that early period

Bourliere: But as you pointed out, that is a rather artificial situation

Engle: Oh, yes.

Andrew: Figure 41 looks very much like the curve showing the number of young water fleas by parthenogenetic reproduction throughout the life span of the mother.

Bourliere: This decline in fertility or egg production, is usually progressive, and the phenomenon of the female climacteric appears to be very specific to the human species. Such a period of vigorous adulthood is of variable duration but is always followed by the period of morphological and functional involution we call senility In warm-blooded vertebrates the maximum duration of the whole life cycle is fairly constant among the various members of the same species if the members are kept in optimal ecological conditions But very few individuals are able to reach the potential longevity of their species The numerous studies made during the last decade on natural populations of wild birds show that the mean expectation of life of a population is always very inferior to the potential life span (7, 8, 9, 10, 11, 12, 13). Senile animals are very rarely found in natural conditions, and the increasing percentage of old individuals is definitely peculiar to our own species and even to our Western civilization

Yesterday, when speaking with one of you, I was asked if this rough outline of growth and aging pattern of warm-blooded vertebrates was true in every case and I was asked about the growth and aging patterns in rats.

First, when speaking of growth, one must always specify which kind of growth we are speaking of growth in weight (ponderal growth), growth in length (statural or longitudinal growth), or relative growth If you have white rats that are overfed in some laboratory colony, you can indeed have a ponderal growth curve which shows a progressive weight increase with age instead of the usual plateau of the maturity period This increase in weight of the older animals is due for the main part to fat deposition, and this condition is very peculiar to rodents kept under laboratory conditions But when you are studying wild mammals—that is to say, species which are living in their normal ecological conditions—you never find such a ponderal growth curve In any case, if you study the statural growth, the growth in length of the animal for instance, you always find that the skeletal growth of rodents comes to its end during the first year of life

Secondly, even among warm-blooded vertebrates, in whom growth is of the determinate type, there seem to exist different growth patterns For instance, among mammals, we have at least the rodent type

and the primate type. Rodents retain during their whole life span some potential for skeletal growth. It has been shown, for instance, that even rats more than two years old can resume skeletal growth, if injected with pituitary growth hormone. In carnivores and primates, on the contrary, the epiphyseal cartilages do not retain their growth potential after maturity.

And finally, before leaving this subject, I should like to point out that it has often been stated that growth, even in wild mammals, is more or less continuous This is due to the fact that in wild populations very few individuals live beyond the beginning of their maturity; most of them die when young or adult. Senility is almost unknown in normal conditions for small mammals and birds and is, so to say, a by-product of domestication and civilization.

The life-table and survivorship curves of the gull *Larus argentatus smithsonianus* banded on Kent Island by Paynter (14), illustrate very well this fact. When the potential longevity of the gulls of this colony, as proved by banding, is of 25 years, the average expectation of life at birth of the members of the very same colony is only of 2 44 years! On another hand, the mortality rate throughout the whole life span remains approximately constant, giving to the survivorship curve a typical "diagonal form " In birds, the maximum mortality rate is found at the egg and chick stages, where it can reach 60 per cent. As soon as the young birds leave the nest the mortality rate settles between 30 and 40 per cent for the rest of the life span. Thus the proportion of "senile" individuals in such natural populations is very low Among small mammals, we find very similar figures Davis (15) found that only 5 per cent of the wild brown rats he studied on a Maryland farm reached the end of their first year of life, against a potential longevity of more than 3 years

The period of senile involution is seldom met with in natural populations. Even in our own species, if we had been studying gerontology, let us to say, two hundred years ago, it would have been much more difficult to find large samples of old people. The increase of the average expectation of life at birth in Euro-American culture during the last 150 years has been tremendous, as compared to what it still is in India and in other overcrowded countries of the Far East. Senility and old-age diseases are true by-products of domestication in animals and of civilization in man

The situation is very different with respect to growth in lower, cold-blooded vertebrates (fishes, amphibians, and reptiles), which are good examples of indeterminate growth (16) Researches made during the last fifty years by the fishery boards of various countries have already shown that fishes have a very prolonged growth period and that growth

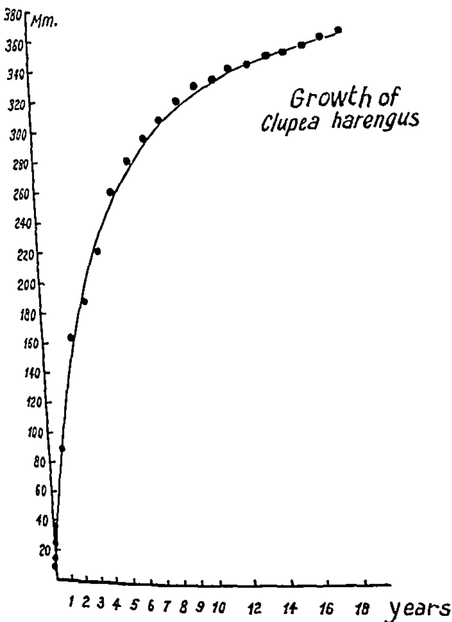


FIGURE 42 A typical fish growth curve Longitudinal growth of *Clupea harengus* Redrawn from Backman (16)

goes on throughout life, slowing down as the animals become older (Figure 42). The scale method of age determination in fishes living in temperate waters, has made possible the study of age-structure and growth of numerous populations of fishes; one of the most striking discoveries resulting from this method is the fact that the fertility of females does not lessen with age, and the longer and older the female, the greater is its capacity to produce eggs. To take a single example, the average egg production per female and per year of North Sea haddocks was found by Raitt (17) to be the following:

31,000 eggs for the 2nd year of life
100,000 eggs for the 3rd year of life
159,000 eggs for the 4th year of life
224,000 eggs for the 5th year of life
278,000 eggs for the 6th year of life

Those are the average results for a homogeneous population, but they show pretty well that the increase in egg production with age is exactly the reverse of what happens to egg production with age in warm-blooded vertebrates.

Andrew: It might be pointed out that in some of the cold-blooded vertebrates there are recent findings of degenerative changes in the gonads, at least in the male gonads, in what would appear to be old age. Dr. Priscilla Rasquin (18), of the Department of Aquatic Biology of the American Museum of Natural History, has published a paper recently on the testes, showing the decrease in the amount of sperm production and the appearance of concretions in the tubules, in a teleost fish, *Astyanax*.

Bourliere: In a few minutes I shall show you the very same thing happening in mosquito-fish.

Engle: Dr Bourliere, what would be the average length of life of the haddock (Figure 42)?

Bourliere: The haddock population in the North Sea has been seriously depleted by overfishing during the last twenty years, and the average expectation of life at the end of the pelagic stage should not exceed four or five years. The oldest fishes taken in the North Sea are eleven years old.

Egg production in old haddocks can reach fantastic figures One Faroe specimen of eleven years of age (the Faroe stock grows more slowly, matures later, and lives longer than the North Sea stock) was found to have 3,295,000 eggs in its ovaries[1]

Gross: In these older animals do you find any increase in pathological lesions, such as tumors, to indicate that there is a—

Bourliere: Up to now there have been very few data on wild fish pathology, especially on the various kinds of degenerative diseases.

For a time my opinion was that tumors did not exist in cold-blooded vertebrates living in natural conditions, but that is wrong. Tumors of various types have been found both in wild fishes and in wild snakes

Lansing: What really determines termination of life in this particular species? Is it attrition by fishermen, so that in the warm waters you described that are closer to the mainland, where there are more fishermen, fish older than 6 years are not found whereas in the colder northern waters, where there are fewer fishermen, there is less termination of life by capturing, and so you find 11-year-old fish, and perhaps in another locality with no fishermen, you would find 20- and 30-year-old fish?

Bourliere: The main cause of death in the North Sea haddock population we are speaking of is human depredation The depletion of the fish stock by overfishing due, since 1927, to the introduction of a new and more efficient type of trawl net is now a cause of concern for the fisheries departments of the various western European countries

Simms: When they are not caught by fishermen, what do they die from naturally?

Bourliere: From natural depredation and parasitism It seems that in every wild population of birds, mammals, fishes, and reptiles, there is a very low percentage of deaths by senility and by degenerative diseases Most of the animals die from depredation, both human and natural. It has been shown that depredation falls principally on very young, very old, weakened and diseased individuals

Simms: In those that don't die from accidents, what diseases do they have?

Bourliere: Various parasitic diseases

McCay: I can't believe that we know the potential life span of these fish I think the only fish for which we know the life span are the carp which were kept in the ponds of the monasteries in which the records go back from one to three hundred years

We kept four species of trout for their whole span of life, and what they die from is fungi infection, ultimately. Where crowding was avoided we got trout up to 4 years of age

I can't believe here that we know anything but a very beginning of the life span of these haddocks, because as soon as an animal under natural conditions, and especially fish, begins to deteriorate slightly, it is subject to fungus disease and to its enemies. So it is eradicated

Bourliere: That is quite right As soon as an individual is weakened by a disease of some sort, it is usually taken by predators

I should like to add a few words to what you said, Dr McCay, on the longevity of carps Flower (19, 20) has made a very careful and critical analysis of the longevity records of verterbrates kept in

captivity. He discusses at length these legendary reports and, finally, admits that the oldest carp *Cyprinus carpio*, which has ever lived in captivity was 47 years old. The fishes which hold the records of longevity of their class are a *Silurus glanis*, which reached 60 years at least, and an eel *Anguilla anguilla* which died when 55 years old

Gross: What is the story with salmon? The female goes to the spawning grounds and lays its eggs, and proceeds to die soon afterwards. What is the cause of death here?

McCay: The Lamprey eels of Ithaca do the same thing. They have a life cycle that runs about four or five years.

Although attempts have been made the cause of death has never been determined The animals finish their life cycle, and they die. If you come to Ithaca about next June and look at the eels, you will see the Lamprey eels dead That seems to be a cycle.

Chow: Dr Hisaw, would it be possible to give these fish some hormones?

Hisaw: Probably what is wrong with them is that they already have had too many hormones. When salmon of the Pacific go up the streams to spawn, they wear themselves out They become exhausted. They don't eat on their way. Reproduction is their last earthly act.

Gross: In the case of the Lamprey eels, they die a year later, is that right?

McCay: No, *if I remember correctly, they are about four or five* years of age. I think they lay their eggs and then die shortly afterwards.

Hisaw: I think it is a similar story, Dr McCay, to that of the Pacific *salmon that is, reproduction is the terminating act of their life*

Chow: What do you think would happen if you kept fish in an enclosure under as natural conditions as we could make, so that there would be as few accidents as possible? Would they live forever?

Bourliere: It seems that *in captivity* most of the wild vertebrates live longer than *in* the wild state *if food* and other *environmental* conditions are good That *is certainly true for most of the wild mammals* and birds You can find in Flower's review and some other papers a lot of data supporting this point of view

In fishes the growth and aging patterns, therefore, appear very different and, in many ways, the exact opposite of those of warm-blooded vertebrates.

But *in* spite of some unchecked suppositions, most fish do not have a very long life span, contrary to the *potential longevity* of reptiles. Numerous observations of field naturalists and zoo keepers suggested that reptiles have a much longer life span and are, therefore, better material *for the study of comparative physiology of aging processes* Available data have been carefully analyzed and criticized by Flower

(21, 22). His data point out the remarkable longevity of turtles, which hold the record of the longest life span among vertebrates and have a very prolonged growth period, as shown by the very interesting data published in 1945 by Flower (23), which I have plotted

In Figure 43 you see the curves for two turtles which were raised in captivity from their first year of life to their fortieth year of life. For each animal there are two growth curves, indicating the growth in weight and the growth in length. The protracted growth period is quite remarkable and characteristic of cold-blooded vertebrates.

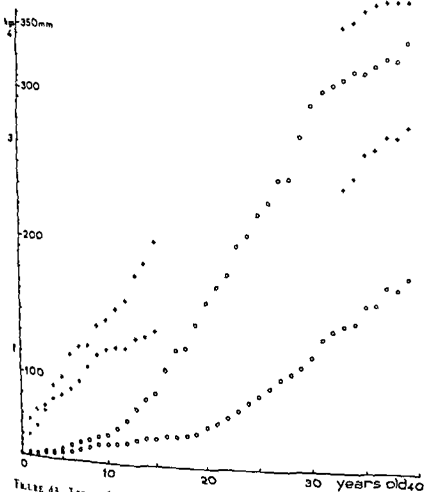


FIGURE 43 Longitudinal and ponderal growth of two turtles *Testudo graeca* [Drawn from Flower's data (23).] Circles indicate ponderal growth and crosses indicate longitudinal growth

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that narrow and more transparent bands correspond to the period of winter cessation of growth, and the broad and less transparent bands to the spring and summer period of intensive feeding.

Such a hypothesis had to be checked. During the last three years

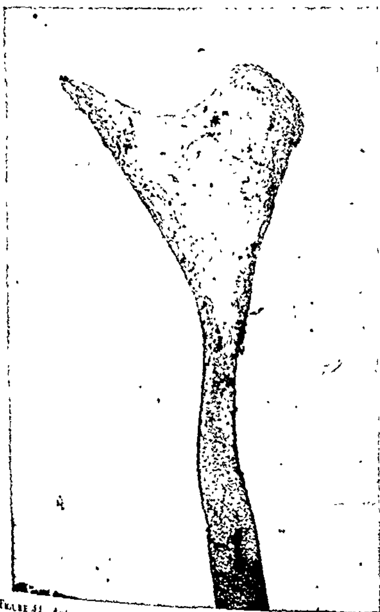


FIGURE 41 Anterior part of the ectopterygoid bone of a newborn *Natrix natrix*

At least five species of turtles are known to have lived more than one hundred years in captivity, and three species at least have reached the ages of 152, 123, and 102 years. Unfortunately, no satisfactory method of age determination for wild turtles has been devised up to the present time, and captive specimens of known age are too scarce to be available for experimental work. The other suborders of reptiles (crocodilians, lizards, and snakes) do not seem to enjoy such a marked ability to become centenarians, but numerous species seem to be able to live to ages of certainly over 20 to 50 years, a very remarkable fact when compared to warm-blooded vertebrates of similar size.

I summarized most of the available data in a paper published in 1946, which gives some of the most remarkable cases (24).

Such a protracted growth period and long life span is not due, however, to the unnatural environment of life in captivity. During the last decade it has been possible to mark snakes for individual identification in the field by clipping the ends of ventral plates in various combinations. Such a method provides data on growth rate and longevity under wild conditions. Without going into too much detail, it may be said that the results obtained by this new method coincide pretty well with what was previously known. The observations made by Fitch (25) on the Californian rattlesnake, to cite but one example, show that growth in natural conditions is rapid in young individuals and gradually slows down as they get older, without evidence of stable adult size. Continued growth was observed in most mature rattlesnakes, but some of the oldest individuals studied by Fitch failed to make measurable growth over a period of a few years

Interesting as they are, these data are unable to tell us very much about the peculiarities of the aging processes in cold-blooded vertebrates. Some method of age determination of free-living snakes needed to be devised to enable us to undertake physiological studies on a large number of animals of known age Without such a method, comparative studies of growth and aging patterns, both in cold-blooded and warm-blooded vertebrates, were impossible. During the past four years I have devoted a lot of time to trying to solve this small problem of comparative gerontology

The first step in that direction was taken by a Russian zoologist, Bryuzgin, who published in 1939 "A Procedure for Investigating Age and Growth in *Reptilia*" (26). Bryuzgin observed on some skull bones of the snakes he investigated, and especially in the anterior part of the ectopterygoid bone, "regularly alternating narrow and wide bands," which he interpreted for the first time as "annual growth rings" Bryuzgin emphasized the correlation between total body length of the animal and the number of what he called "winter rings." He believed

that narrow and more transparent bands correspond to the period of winter cessation of growth, and the broad and less transparent bands to the spring and summer period of intensive feeding.

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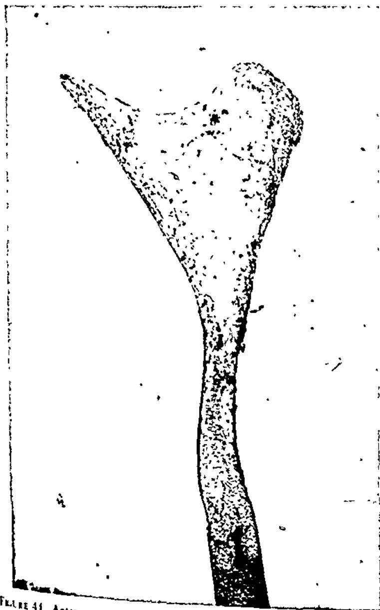


FIGURE 41 Anterior part of the ectopterygoid bone of a newborn *Natrix natrix*.

I reinvestigated the problem and modified slightly Bryuzgin's method. Instead of "clearing" ectopterygoid bones in glycerine I was led to use water in young animals, and xylol in older ones. Such "cleared" bones are examined under a low magnification microscope, and drawings or photographs are made of the anterior part of both ectopterygoid



FIGURE 45 Anterior part of the same bone of a 10-12 year old *Natrix natrix*, showing the regular alternation of thin transparent winter bands and broad and opaque summer bands.

bones. "Winter bands" may be found in most of the other skull bones, but nowhere else are they so easily detected. Great care must be taken to count the thin transparent and regular "winter bands" and not the broad and opaque "summer bands"; winter bands indeed must not be confused with less regular bands that may be found right in the middle of the summer bands, and which are probably due to a temporary period of estivation or to a change in the calcium metabolism at the

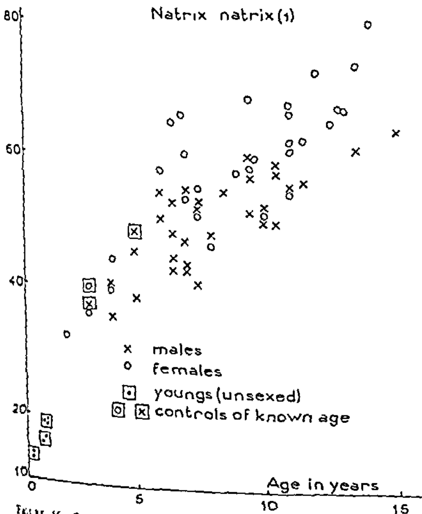


FIGURE 46 Growth curve (body length in cm) of a population of *Natrix natrix*. Circles indicate males and crosses indicate females. Body lengths of controls of known age (boxed circles, crosses and points) agree fairly well with body lengths of snakes whose age was computed by the 'animal rings' method

time of egg laying in females. Our material consists of two common species found all over western and southern Europe: the common European grass snake (*Natrix natrix*) and the viper (*Vipera aspis*). The first species, being non-venomous, is now preferred for routine work.

Figure 44 is a photograph of the anterior part of the ectopterygoid bone of a newborn grass snake. No zone of differential deposition of bony material is apparent.

In an older snake of the same species, the same bone looks quite different as shown in Figure 45 Broad dark zones are clearly visible and are separated by narrow and more transparent winter bands. In any case, the count of winter bands must be made on ectopterygoid bones of both sides, and on the internal and external sides of each bone

Our next step was to be sure that the so-called winter bands were actually produced in wintertime and were actually yearly marks, comparable to the annual rings on the fish scales. For this purpose I compared the body length of my snakes whose age was computed by the method just described with the body length of snakes of the same species raised from the eggs in an open-air terrarium, and whose age was thus accurately known As shown in Figure 46, the correlation is good, and the body lengths of my snakes correspond pretty well with those of snakes of known age

Bryuzgin's method is thus an efficient means of age determination for temperate zone snakes and enables us now to divide snakes taken in the field into a number of age classes

The few data gathered during that first phase of our research do not enable us to draw any definite conclusion. Nevertheless these preliminary investigations raise some interesting questions and suggest some tentative hypotheses.

In the first place, our data show that the growth pattern of snakes is quite similar to that of fishes and other cold-blooded vertebrates studied up to now One must note that the growth is very rapid in the first five years but that afterwards the growth rate remains very high, even in snakes 15 years old (Figure 46). The rate of growth of females is definitely more rapid than in males, and such a result agrees very well with earlier observations showing that in the common grass snake old females may be twice as along as old males.

Our observations on longevity also confirm what was previously known on the life span of other snakes· Flower's longest-lived specimen was an Anaconda, 29 years old, but I know of a *Vipera aspis* raised from the egg by P. Roux and now 25 years old, and still in good condition. My oldest grass snake is more than 15 years old, and it is fair to suppose that the potential longevity of that species is over 20

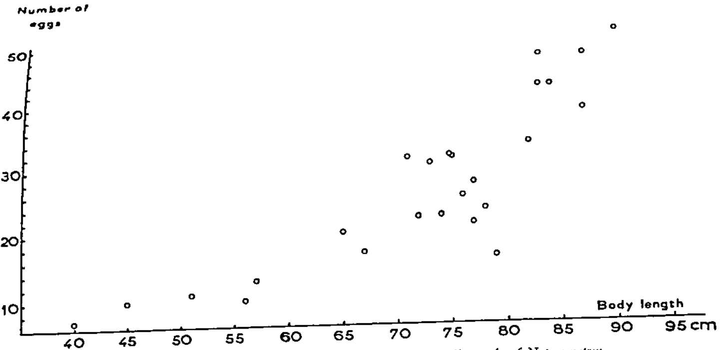


FIGURE 47. The increase of egg-production with age in a small sample of *Natrix natrix*.

years. One female is known to have reached a total length of 174 cm, and one male a length of 107 cm., but such giant and probably very old specimens are very scarce in natural populations.

The variation of egg production with age seems to be a promising field of investigation in snakes. Our data are not very numerous at the present time, but scanty as they are, they nevertheless show a definite increase of the egg production with body length (i.e., with age) (Figure 47). In *Natrix natrix* the clutch size varies with age from 16 to 51 eggs per clutch, and our data for another species, *Coronella laevis*, show the same trend (from 4 to 13 young per brood in that viviparous species).

From these observations it may be concluded that snake fertility, like fish fertility, increases regularly with age; senile infertility, so characteristic of very old warm-blooded females, appears to be unknown in cold-blooded vertebrates, thus emphasizing the basic dissimilarities of both their growth and aging patterns. Such a conclusion must nevertheless be supported by a greater number of observations.

The next graph (Figure 48), redrawn from Klauber's data (27) shows that there is a slight decrease in fertility in the longest (oldest) female rattlesnakes investigated. These data suggest that there is a slight decline in fertility in the oldest female rattlesnakes. Such a fact is also supported by a few observations made on fishes Even in the haddock, of which I was speaking at the beginning of this session, Raitt (17) found that "there is some evidence of a slight decline in egg production" in the oldest females On the other hand, Krumholz (28) studying the small, short-lived and viviparous *Gambusia affinis affinis*, found a "period of sterile senility following reproduction." As a matter of fact, the period of reproduction in female mosquito fish embraces only a single series of broods, and it has been shown that the size of broods becomes smaller as the reproductive period progresses, whereas females continue to grow until they die.

The study of the quantitative and qualitative variations with age of various organs and tissues has been undertaken, and up to now no definite senile involution has been found Organ weight is, on the whole, closely correlated with total body weight But it may be pointed out, once more, that senile animals are always very rare in natural conditions; our data tell us that as long as growth goes on, the various organs continue their development—but no more If our animals were allowed to live undisturbed in captivity, maybe they could reach an age where growth would cease, and after which morphological and functional involution of their organs could start In fact, testes weight of our two oldest males is slightly inferior to the weight of those of animals two or three years younger Volsoe's data (29) on the varia-

FIGURE 48 The increase of egg-production with age in a large sample of Californian rattlesnakes. Note the slight decrease in egg-production in the oldest females Drawn from Klauber's data (44)

years. One female is known to have reached a total length of 174 cm., and one male a length of 107 cm., but such giant and probably very old specimens are very scarce in natural populations.

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anisms implies frequent stresses both for our nervous and endocrine systems to keep constant the temperature of the internal medium; and one may assume that such continuous adaptations speed up aging processes It is interesting to note, from this point of view, that what is now called in continental Europe "artificial hibernation," where a pharmacological block of the autonomic nervous system is associated with refrigeration of the patient, bringing his central temperature down to 29° C. (about 85° F) enabled Sénèque and Laborit* to perform surgical operations in decrepit old people, who would otherwise have been unable to support surgical procedures

But it must not be understood that the working of thermoregulating mechanisms is responsible alone for speeding up the processes of aging in warm blooded vertebrates It is now well understood that the transition from poikilothermy to homeothermy is achieved by a fundamental change in the metabolic rate of the tissues themselves. The basal heat production of the homeotherm is considerably greater than that of the poikilotherm of similar size as shown by the following figures (Table X) borrowed from Davson's recent book (35).

To sum up, warm-blooded and cold-blooded vertebrates have very different growth and aging patterns These differences, moreover, appear to be more differences of tempo than basic differences of mode, as shown by the intermediate condition of some lower mammals. The slowing down of metabolic processes which is the result of true hibernation is probably responsible for most of the differences observed but, on the other hand, the metabolic rate of the tissues at the same temperature is quite different in poikilotherms and homoiotherms So, it would be very important to test further at the tissular and cellular level and to try to understand the causes of this difference in tissue metabolism

TABLE X

Heat Production of Poikilotherms and Homoiotherms of Approximately the Same Weight, at 37° C.

Poikilotherms	Weight (Kg)	Cal / 24 h	Homoiotherms	Weight (Kg)	Cal / 24 h.
Python	32	189	Man	32,2	997
Alligator	53	408	Man	53	1470
Tortoise (flesh)	117	876	Man	109	2559
Boa	10	55	Dog	11,5	389

*Unpublished data

tion of testes length with total body length in *Vipera berus* similarly show that in the oldest males, which are extremely rare, "the testes do not follow the growth of the animal."

Such observations would lead us to think that the aging processes are probably not so different in poikilothermic and in homeothermic vertebrates, as was previously supposed. The difference would be more a difference of tempo than a difference of mode. The lack of temperature regulation in lower vertebrates enables them to live, so to say, more slowly, thus slowing down the processes of growth and aging

Comparative physiology of mammals support strongly such a point of view. Thermoregulation is very unequally developed even in the various orders of mammals, and it seems that the life span varies accordingly. In this respect, bats (Chiroptera) are a very favorable material for our studies. The very long life span of captive frugivorous species has already been pointed out by various authors (30). But the average expectation of life at birth of small free-living insectivorous bats is far beyond that of other mammals of similar size. In 1947 (31) I reported the case of a *Rhinolophus hipposideros* banded as adult in October, 1938 and still living in fine condition in May, 1946. Similar observations have been made by other zoologists, and *Trapido* (32) published a longevity record of twelve years for a captive vampire. Recently longevity records of 13 years for a *Myotis myotis* and of 13½ years *Rhinolophus ferrum-equinum* have been reported by Bels (33).

Now, it has been shown that bats have a very poor thermoregulation even outside the hibernation period Pearson (34), investigating the rate of metabolism of some small mammals, found that this metabolic rate was very low during the daytime, almost at the hibernating level. During about twenty hours a day, these little bats have a very low oxygen consumption. On the other hand, bats have a very low fecundity, usually one or two off-spring a year Inversely, shrews, which are among the shortest lived mammals, have both a very high metabolic rate and a very high fecundity Undoubtedly, bats and shrews are very interesting species from our point of view, and I hope to start in the near future a study on the variations of tissue metabolism of these lower mammals It must be added that in birds, the Trochilidae (hummingbirds), seem to have a metabolic pattern very similar to that of insectivorous bats.

All these observational data appear to indicate that, among vertebrates, a limited life span and a limitation of growth potential is, so to say, the counterpart of the homeothermy of the internal medium. Thermoregulation obviously enabled the warm-blooded vertebrates to thrive in climate zones where life would otherwise be impossible for cold-blooded animals. But the existence of thermoregulating mech-

with some of the observations that have been reported I don't think we have such information.

Engle: Well, there is a rather interesting corollary here: in cold-blooded animals, linear growth and egg production will increase according to a more or less similar logarithmic curve throughout life span, whereas in the [illegible] at the [illegible] egg p[illegible]

Hisaw: The thing, though, that we must keep in mind is there are two or three things in an animal's life that we are considering here and which are not necessarily tied together For example, growth and reproduction. The important thing with respect to the growth of the animal and its aging is that it live long enough to produce viable eggs and sperm for reproduction That is the first essential step. Beyond that, it matters little for the survival of the species as to what might happen to the individual animal In some animals, as in the salmon and in the lamprey, which have been mentioned, and we find many examples in the invertebrates, the reproductive act is the terminal act of the animal's life that is, they live that long, and that is the end In other words, in those animals reproduction and aging, or the age limit, are closely linked up with one another

Now, in reptiles and amphibia and certain other animals, as has been mentioned, that is not necessarily true Reproduction in such animals is not limited by or necessarily correlated with definite stages of growth and aging

Bourliere. A good example of what Professor Hisaw is saying is the case of the mosquito fish, a short-lived species, in which there is a decline in egg production with age, but no cessation of growth

Frank Is there a difference in the capacity to mobilize and retain calcium and phosphorus in these animals? I wondered whether there has been any attempt to use tagged atoms

Bourliere I don't think there has been any experimental work done on that

Frank. In this continued growth, as contrasted with that of the warm blooded human, which reaches a certain stage and then maintains a sort of equilibrium of taking in and letting out, there is more taking in than letting go, in order to continue to grow. I wondered whether there was any clue to this difference in metabolic rate as part of the dynamics of this continued growth. Also, these reptiles have to continue laying eggs, which means more requirement for calcium

Simms I am very much interested in the question of life span in different species I am referring to the life span as limited by disease or endogenous causes rather than by trauma or accidental deaths or

Engle: Well, Dr. Bourliere, I have been fascinated with this presentation of yours from three aspects: (*a*) linear growth; (*b*) ponderal growth, and (*c*) the correlation in those two growth factors with egg production.

I should like to ask a question about this linear growth. In cold-blooded vertebrates and in the rat you are dealing with animals which, to the best of my knowledge, do not have epiphyseal closure that is dependent upon sex steroid hormone production. Could you amplify that a little bit? I am unfamiliar with this field of bone growth in the lower vertebrates.

Bourliere: I don't know of very much work done on that subject. It seems that in most cases the bone growth goes on throughout the life span, but what is very curious is that the pattern of bone growth in cold-blooded vertebrates is much more, so to say, plastic than in warm-blooded vertebrates. The experiments made in this country by Gabriel (36) on fishes and by Fox (37) on snakes seem to indicate that both the number of vertebrae and the scutellation may be affected by environmental differences of temperature during the first stages of the development of the animals.

Engle: Well, that is all very interesting Of course, that might be just the critical period. We know that every developmental organ has a critical period in which alterations of its metabolism will cause abnormalities to appear.

But going back to linear growth, in your reptiles you get a linear growth throughout life. We think that in the higher mammals, including man, the epiphyseal closure starts, and linear growth is limited by the development, in adolescence by the steroid hormones

Now, is there any such sort of thing in the cold-blooded vertebrates? Is there any approximation of a steriod hormone that could act on an epiphyseal plate and, through the pituitary mechanism, cause a closure of that epiphyseal plate?

Hisaw: I think it is perfectly true, as has been mentioned by Dr Bourliere, that information with respect to the physiology of cold-blooded animals nowhere approaches our knowledge of mammals

Our studies of cold-blooded animals usually have been rather short-ranged and not carried on over a long period of time under conditions that would bring out the results that you have in mind. The long-range experiments have usually been of a different nature For example, tadpoles were hypophysectomized, as P E Smith did many years ago (38), and retardation of growth observed, and then that was topped off by a rather acute experiment at the end to see if the animals retained their ability to respond Such experiments did not go into the matter of prolonged growth, so they did not give suitable data for comparison

with some of the observations that have been reported. I don't think we have such information.

Engle: Well, there is a rather interesting corollary here· in cold-blooded animals, linear growth and egg production will increase according to a more or less similar logarithmic curve throughout life span, whereas in the higher mammals, egg production comes to a peak, say, at the time the epiphyses are closed and linear growth ceases, and then egg production goes through a typical bell-shaped curve and declines.

Hisaw: The thing, though, that we must keep in mind is there are two or three things in an animal's life that we are considering here and which are not necessarily tied together. For example, growth and reproduction The important thing with respect to the growth of the animal and its aging is that it live long enough to produce viable eggs and sperm for reproduction That is the first essential step. Beyond that, it matters little for the survival of the species as to what might happen to the individual animal In some animals, as in the salmon and in the lamprey, which have been mentioned, and we find many examples in the invertebrates, the reproductive act is the terminal act of the animal's life: that is, they live that long, and that is the end In other words, in those animals reproduction and aging, or the age limit, are closely linked up with one another

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Sterns. I am very much interested in the question of life span in different species I am referring to the life span as limited by disease or endogenous causes rather than by trauma or accidental deaths or

being devoured by other species. As far as I know, the life span in *all species, certainly in humans, in rats and in drosophila, is limited,* first, by the intrinsic susceptibility to disease at birth, and second by the rate at which the susceptibility to disease increases with advancing age, or, in other words, the rate at which lesions or pathological conditions develop with advancing age.

In Figure 49 you will see a plot of the logarithm of the probability of death against age (39). In using the term "probability of death" I am speaking about the number of deaths per year in a given age group divided by the number of individuals reaching that age group.

For total deaths of humans we have the curve as shown. At the left are the infant deaths. Then there is a slight hump due to tuberculosis. After that the curve rises as practically a straight line—represented by the equation $kt = \log P_t - \log P_o$ where t is age, P_t is the probability of death at age t, P_o is the extrapolated probability of death at birth, and k is a constant.

If we consider only the predominant lethal diseases of adults, we can extrapolate this line back to zero age. We call this point P_o. This value *represents the intrinsic probability of death from the adult diseases at the time of birth.*

For rats the value of P_o is only slightly higher than that for humans, but the curve goes up very steeply The short life span of rats, as com-

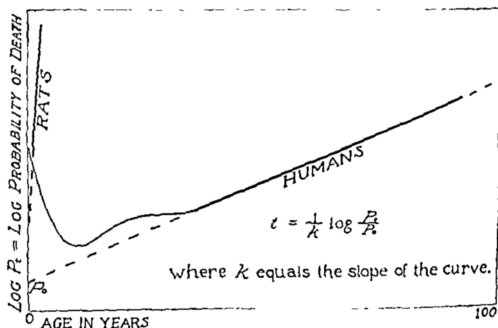


FIGURE 49. Human and rat mortality curves.

pared with humans, is due to this rapid increase in the mortality rate within a very short time. That is the result of rapid accumulation of lesions of various types in the rat.

With drosophila there is a similar curve which would be almost a vertical line on this chart.

Now we come to the question of other species. As is obvious from the data that you have presented, in general the larger animals have a longer life span than the smaller animals. There are exceptions to that, but I think as a general rule that is true.

Would you have any suggestions as to what factors might be involved in causing that? In other words, larger animals have a slower rate of accumulation of lethal lesions than do smaller animals. Why should there be that difference?

Bourliere: No doubt, the comparison of the types of survivorship curves calculated for various animal populations raise a lot of interesting questions, as shown by E. S. Deevey in his 1947 paper (40). But I wonder whether the samples on which most of these life tables are based are big enough to enable us to draw curves as reliable as those we have for various human populations, for white rats and even drosophilas. Before going further in the causal analysis of their differences, we need to gather more numerous data.

Concerning the comparative longevity of large and small animal species, it is obvious that, both in cold-blooded and in warm-blooded vertebrates the larger animals have a longer life span than the smaller ones.

Himwich: It may be related though to the metabolic rate of the tissues. In general it is known that the smaller the animal the greater the oxygen consumption per gram of tissue. The life span may be an inverse function of that relationship, the greater the metabolic rate of the tissue the shorter the life span.

Simms: That may be so and is a possible explanation. But can you explain why a faster metabolic rate would cause tumors and other lesions which limit the life span to appear much earlier and to accumulate much faster?

Himwich: Of course, the fact is that more cancers appear in old age as the metabolic rate decreases. Whatever the reaction may be to tumor growth the general point that Dr. Bourliere made was that the greater the metabolic rate the shorter the life span.

Shock: If you plot the *mortality* data for snakes in the dimensions of Figure 49 you would get a line passing through P_o parallel to the X axis, wouldn't you?

Simms: No, because the snakes finally die without reaching extreme ages.

being devoured by other species. As far as I know, the life span in all species, certainly in humans, in rats and in drosophila, is limited, first, by the intrinsic susceptibility to disease at birth, and second by the rate at which the susceptibility to disease increases with advancing age, or, in other words, the rate at which lesions or pathological conditions develop with advancing age.

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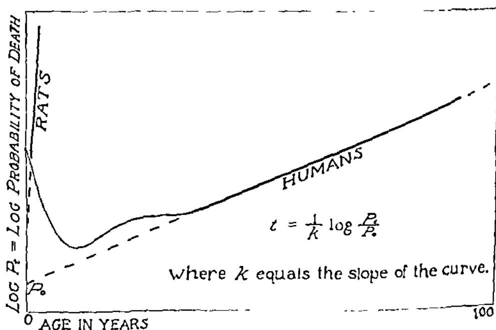


FIGURE 49 Human and rat mortality curves

of time in a tissue culture when you go from 31° C. to 37° C. (41). In the case of rats, the isothermal temperature for the animal is very close to the limit of tolerance for their tissues You can't go much above 44° C. and expect cells to live. On the other hand, the isothermal temperature for those tissues in the natural host is about 39° C Thus, your differential is very low

Animals that have a long life span can go into a state of dormancy. During these intervals there may be great recovery, and with this, perhaps great reserves are stored up. On the other hand in animals with a high metabolic rate, the limits on reserves are very low, and one could expect disastrous conditions to appear at times

In the case of fishes, on the other hand, I am led to think of the great abundance of trace elements in the sea, and the low temperatures which tend to reduce activity to a minimum. I wonder what would happen if these carp, for example, were kept at an elevated temperature where the incipient effect of heat on the tissues would greatly alter their physiological competence

Hoskins Is there anything known about the life span of animals of a given species living in the northern regions as compared with those, say, living at the equator or where they are subject to high temperature a large part of the time?

Lansing: We don't have data on animal species in their natural habitat but Loeb and Northrup (42) maintained drosophila at different temperatures under laboratory conditions Increased temperature effected a short life span, and a decrease in temperature, an increase in life span

Bourliere: The data on the comparative life span of temperate and tropical species are quite scarce now, at least for the vertebrates. But there is some indication that cold-blooded vertebrates grow slower, mature later and live longer in temperate regions than in the tropics

A few years ago, R B Miller (43) compared the growth curves of two subspecies of the fish *Thymallus signifer* inhabiting two different climatic zones The subarctic subspecies *Thymallus signifer signifer* of the Great Bear Lake, Canada, had a greater average longevity, a later sexual maturity and a slower growth rate than the southern subspecies *Thymllus signifer tricolor*, of Ford Lake (U S A), which live in less cold waters

For warm-blooded vertebrates, the scarcity of data does not permit us to draw any definite conclusion Nevertheless, the tropical species living in captivity are quite comparable, from the longevity point of view, to temperate and sub-polar species of similar size

Concerning the invertebrates, the hot tropical environment seems to be able to speed up, in most cases, the life cycle of various insects

Shock: But the rate of death does not increase with increasing age.

Simms: Are you sure it doesn't?

Shock: That is what Dr. Bourliere's data seem to say.

Simms: I thought there were data to show that it does increase.

Bourliere: As far as we can conclude from the available data, the mortality rate for snakes seems to remain constant throughout life, but one must not forget that disease plays a very indirect and a comparatively small role in that mortality.

Shock: The chances of a 19-year-old snake living to be 20 years is just as good as the chances of a 2-year-old living to be 3 years, isn't that correct?

Bourliere: It seems so.

Simms: If the curve did not rise some, you would have snakes living thousands of years, and of course, there is no evidence for that.

Shock: Not at all. The line parallel to the X axis says only that the probability of death at any given age is constant. As long as there is some probability of death the individual would ultimately succumb. Constancy of death rate is not equivalent to immortality

Bourliere: It is quite possible that the potential longevity of reptiles is actually much greater that their maximum longevity in natural conditions. The extreme longevity of some captive turtles is a good indication of that The survivorship curves of cold-blooded vertebrates we have are those of *natural* populations, living in conditions where predation is the major cause of death, and where most individuals die before having any chance to get old.

Simms: We do have the giant tortoise living several hundred years, but the life span is much shorter for most species In my plots if a species had a horizontal line it would have an almost indefinite life span

If I am not mistaken Dr Bourliere and I are using the term "mortality rate" in two different ways My use of the term refers to deaths in a given age group relative to the number reaching that age group I think Dr. Bourliere is speaking about the deaths in an age group relative to the total animals of all ages That would account for the apparent difference in our conclusions

Gey: Don't you think that some of this might be related to the incipient effects of heat on the tissues of the organism? If an old snake were forced to stay at an elevated temperature for a while, it is quite possible that he might succumb to some infection or simply run downhill whereas if he had a choice of going to a cooler place, he would make himself a lot more comfortable.

I could illustrate, in terms of tissue cultures, how dramatic is the effect of temperature on, let us say, the pH reduction over a period

which is intercellular and itself non-vital in animals of different species and ages

Bourliere: This problem of body size is a very difficult one As it has already been pointed out in the discussion, larger animals have, on the whole, a longer life span than the smaller ones. That seems to be true for the insects too, even for different castes of the same species

Among the social insects, bees, wasps, ants, termites, we find various castes integrated in what has often been called a "super-organism," i e a very complex social structure In ants and termites, the queens, i.e. the reproductive females, are in most cases bigger than the individuals of the other castes, especially workers and soldiers. These queens are, on the other hand, true egg laying machines and one could expect that such an intense metabolic activity would be correlated with a short life span. As a matter of fact, it is just the reverse. Termite queens are very probably able to live up to ten years and more, and the queens of some species of ants at least seem to enjoy a very remarkable longevity Thanks to Dr Le Masne*, myrmecologist at the Sorbonne, I can present you some actual figures: a queen of *Stenamma westwoodi* lived 5 years, a queen of *Formica rufibarbis* lived 5 years, some queens of *Formica fusca* reached the age of 15 years and queens of *Formica rufa* are said to reach 20 years

Coudry: How long do the males and workers live?

Bourliere: The life span of the males and of the workers is always much shorter than that of the queens, from some weeks to some years, according to the species considered

Himwich: Have you estimated the metabolic rate of the queen ant as compared with that of the workers?

Bourliere. I never heard of such a determination

Himwich. It is possible that the queen has a low metabolic rate in comparison with that of the workers. Determinations of metabolic rate of both kinds of insects could throw light on this problem

Carlson. My understanding is that the working bee is a female, not the male, is that right?

Himwich That is right

Bourliere In *Apidae* and *Vespidae*, the workers are generally non-reproductive females Is it not true, Professor Hisaw?

Hisaw Yes, they are females

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*Unpublished data

In sub-arctic and temperate zones the entire life cycle of the butterflies of the family Pieridae is known to have an average duration of one year. In the tropics the same life cycle can be shortened to less than one month. Two males of the species *Appias lyncida* have thus been observed by Fountaine (44) to reach the adult stage in only 17 days. The duration of the egg stage was 2 days, the length of the caterpillar stage 8 days and the one of the chrysalis 7 days. The duration of the adult stage was not measured, but from what we know of other species in similar environments, its duration probably did not exceed two weeks

Hoskins: This brings us back to the old question which has been debated at various other times· are we dealing with a matter of subtraction or a matter of addition? As the individual grows older, has he exhausted some necessary elements in his system or has he accumulated deleterious elements?

Simms: You mean whether aging man has lost a vital function or acquired a toxic or lethal factor?

Hoskins: That is about what it amounts to. That might be a factor, as well as the accumulation of lesions, to which you have referred

McCay: While we are discussing fish [illegible] not [illegible] of fi[illegible] and customs and conditions of t[illegible] species is very great In addition there are wide differences in anatomical characteristics as for instance the very short intestinal tract of the trout and the long tract of the carp

And also when we are talking about indeterminate growth in fish, it seems to me we have to remember that there are many fish that seem to have very determinate growth. We tend to plot a very regular curve, such as you have shown in Figure 42, but it gets all tied in with reproduction, because with the growth of trout, you have each year a period of about seven months when there is substantial growth and increase in body weight. This is followed by a five-month period, which is the reproductive period, when there is little or no increment in weight; when muscle tissue is converted into egg and sperm

So, really, the growth curve is never uniform, even in so-called indeterminate growth. There is a period of plateau and a period of rapid growth, each year. And then each year that period of rapid growth slopes off more towards the horizontal, so in the end you might ask yourself, if your trout survive long enough, whether there is such a thing as indeterminate growth, because every year the slope is decreasing and becoming more nearly parallel to the X axis.

Cowdry: I should like to ask whether the size of the body is a determining factor in longevity. I wonder whether there are any rela-

Chow: What is that ratio?

Simms. The ratio of the *k* values for the two species is 31 to 1. In other words the rat death rate increases 31 times faster than the human death rate.

Lansing: It seems to me we can use the ecologic approach to very good advantage in getting at some of these problems that have been plaguing us for so many years, such as, is the limitation of span of life completely a product of environmental factors, degradation, degenerative diseases, infectious diseases, and so on, or is there also a built-in limiting factor to longevity, or span of life? I think we can get at that by not using a statistic, as Dr Simms did, but rather simple survival curves

Simms: A survival curve is another way of representing the same data.

Lansing: Yes, but I think we can be more the naturalist and use the ecologic data in the raw form to get some information

We can plot span of years, or days, whatever we wish, along the abscissa, and along the ordinate, the per cent of survivors of the initial population.

If we take an ideal situation, which, of course, does not exist, one in which there are no degenerative diseases, no infectious diseases, no predation, and no built-in aging, our population will begin at 100 per cent, of course, and persist to infinity at 100 per cent (Curve 1, Figure 50)

Let's take a hypothetical situation in which we have no degenerative disease, none of the environmental factors, but a built-in aging factor. Then our population would persist at 100 per cent through birth, through adolescence, through maturity, and so on, and at some arbitrary point drop off immediately with no variation if our genetic constitution is controlled (Curve 2, Figure 50) In the absence of genetic variation and environmental variation, we will then, through inherent senescence, have all the animals dying at some arbitrary time. the human, let's say, at 100 years, the rotifers at 30 days, and so on This is, of course, very hypothetical

Actually what we have in man, in drosophila, in rotifers, in paramecia, with determinate or indeterminate growth, is a curve that is not unlike a sigmoid curve (Curve 3, Figure 50)

Now, what does this mean in effect? If we impose, let's say, the human childhood diseases, polio and so on, upon the youngster in his early years of life, we have a certain number of deaths, and so we have a drop from 100 per cent survival in our survival curve And let's assume we have no other causes of death, and no senility. Then that population, having that one factor operating early, will now persist

Bourliere: But not reproductive females.

Hisaw: No. But what can happen in a colony if, say, the queen is killed by some unfortunate accident, is that one of the young workers larvae that has not gone too far in its development, can be changed over into a queen. They are genetically female.

Chow: How do they choose which bee is to be the queen, and how does it change?

Hisaw: It is a matter of diet. If the young animal is fed royal jelly, a particular diet, it changes the potential worker into a queen, and they also give her a different cell, a queen cell, in which to grow.

Chow: I just wonder whether that ratio is equivalent to the ratio of average life span of humans to that of rats.

Simms: If you are referring to the "life expectancy," the ratio of rats compared with humans is from 1 to 33 which is a slightly greater difference than the k ratio. The equation for life expectancy is:

$$t = \frac{1}{k} \log \left(\frac{1.6k}{P_o} + 1\right)$$

Since the life expectancy is not a linear function of k, the answer to your question is "No." However, it so happens that the relationship you suggest is not very far off in this instance. This is because the value of (k/P_o) is roughly the same for these two species. This is probably a coincidence

Shock: Dr. Gardner and his collaborators have shown that the feeding of royal jelly to drosophila increased their life span considerably (45). I believe the increased longevity was attributed to the pantothenic acid content (46). The same authors had previously shown an increase in the life span of mice following the addition of yeast nucleic acid in the diet (47)

Chow: Dr Simms, do you think these two curves (Figure 49) will coincide or more or less coincide if you put the logarithm on the ordinate, as you have, and on the abscissa, plot the per cent of the average life span?

Simms: You can adjust the X axis and the Y axis so that the two curves will coincide.

Chow: Can you adjust it by plotting it as percentage of the average life span?

Simms: No, that would not bring them exactly together. It would come close to it, however.

Chow: How can you bring them together?

Simms: You can do it as follows: First divide P_o for the rat by a constant (in this case 21) to make it equal to P_o for humans Then multiply the rat ages by the ratio of the k values (the slopes of the curves) for the two species.

Chou What is that ratio?

Simms The ratio of the k values for the two species is 31 to 1. In other words the rat death rate increases 31 times faster than the human death rate

Lansing It seems to me we can use the ecologic approach to very good advantage in getting at some of these problems that have been plaguing us for so many years, such as, is the limitation of span of life completely a product of environmental factors, degradation, degenerative diseases, infectious diseases, and so on, or is there also a built-in limiting factor to longevity, or span of life? I think we can get at that by not using a statistic, as Dr. Simms did, but rather simple survival curves

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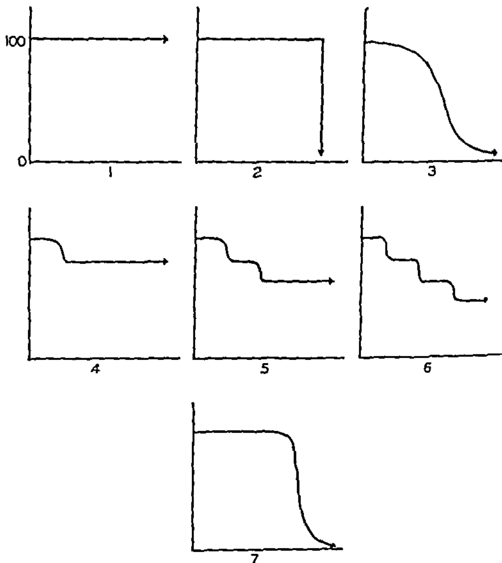


FIGURE 50 *Curve 1* Hypothetical survival curve of a population free of inherent aging, disease, and environmental deaths In effect, an immortal population. *Curve 2* Hypothetical survival curve of a genetically homozygous population free of disease and environmental deaths but experiencing death by inherent aging. There is 100% survival until all animals die simultaneously of aging *Curve 3*. Sigmoid nature of a typical survival curve which implies that many factors operate to attenuate a population *Curve 4*. Hypothetical survival curve of a population which is free of all causes of death except childhood diseases The survivors of the childhood diseases would go on to live indefinitely. *Curve 5* Similar to Figure 44 but adolescent diseases are added to produce a second drop in survivors but the remaining individuals go on to live indefinitely. *Curve 6* Similar to Figures 44 and 45 but adult causes of death such as heart disease are added to produce a third reduction in the number of survivors. Here again any survivors should go on to live indefinitely

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ad infinitum at, let's say, 90 per cent survival (Curve 4, Figure 50).

Now, in the same population, let us say that there are certain adolescent infectious diseases that traumatize that population further, and we drop down another amount. That population still goes on, assuming we have no built-in aging factor (Curve 5, Figure 50).

Finally, if we include coronary diseases and other degenerative diseases, we get a further reduction in the number of survivors in the population, which then can theoretically take us to extinction of the population if enough degenerative factors exist to wipe out the population (Curve 6, Figure 50)

[illegible]

nate in extinction of that population at some arbitrary time or whether we have superimposed upon a pattern such as this, another factor, inherent aging, which finally decimates those late in life who have survived these various infectious and degenerative diseases.

Among the rotifers that were raised under highly standardized conditions—to the best of our knowledge in the absence of infectious disease, with highly standardized nutritive conditions, with no genetic variations, longevity, (the span of life or survival) stayed at 100 per cent through the definite phases of the life span (48) There were no deaths at all in the population at early ages Then very abruptly, approaching the ideal situation, almost all of the animals died (Curve 7, Figure 50)

Of course, there is some scatter in the average curve This is still biological material, and working with short time units and single-day measurements, one can't get the ideal But this is all suggestive that if one can eliminate infectious diseases, degenerative diseases, one still has a factor operating which limits life span, and I think that is what we mean by aging Aging is that which is left over to cause termination of life span when the environmental factors are removed.

Gey You can't say that your environmental factors are completely removed here, because in the case of certain virus diseases you have long incubation periods The examination of these organisms for cellular lesions would be a most important thing in determining what breaks down first

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unless other diseases kill off the survivors or unless the last survivors do succumb to inherent aging *Curve* 7. Survival curve of a typical population of genetically homozygous rotifers grown under highly standardized conditions There are no deaths during infancy, adolescence or adulthood Most of the animals die abruptly at an advanced age to yield a survival curve similar to that of Figure 42

Lansing: Yes, I know. I am aware of the fact that you are rather intrigued by the possibility that viruses may be operative in aging. One cannot by any means write that off or short-change the possibility.

But again, if I can invoke the rotifer data, you will remember that we found we could transmit an aging factor through the egg from older mothers, with shortened life span to the offspring The offspring would carry it through its life span, as would the eggs taken from it late in life, and the second generation would have its longevity still further reduced. If we continued this for a long period of time, you would then say that we were building a virus concentration in this special line of rotifers with old mothers. But at any generation in that series, third or fourth, which now has an attenuated life span or accelerated aging, if we reverse our procedure to selection of eggs from adolescent mothers, which, according to you, would have a high virus titer, we abruptly get a reversal in the trend and an increase in longevity. That is not consistent with the concept of—

Gey: You might be selecting in favor of resistance to something by doing so.

Lansing: There is no selection, because the sisters of the same mothers on different days of life are also followed and give the diametrically opposed picture in regard to longevity

Frank: I am interested here because it seems to me that we are again back where we have been many times before What are the criteria which we are going to use in studying aging? Some people prefer a structural one, some a pathological one, some a biochemical one We have batted this around many times before

I don't think we can or should attempt to legislate on this question and try to tell different people how to do it But if we are genuinely concerned with the processes of aging and recognize that you cannot directly observe a process but can only infer it, then our question is, "What inferential value are you going to give to different kinds of observations?" That is why I am genuinely concerned about formulating a theory of aging that recognizes the dynamic processes by which a living configuration maintains itself over a period of years by this continual transactional intercourse with the environment, as I suggested some years ago (48).

I think it might be worth while to emphasize here this concept of continuity and persistence, but persistence only through continual change Here we consider a dynamic concept as against a static one, as we realize that nothing in this universe can maintain itself unless there is continual activity and intercourse with its environmental field

We have some rocks, crystal structures that have persisted from the Pre-Cambrian apparently unchanged. We also have some radioactive

elements which have been progressively aging, if you please. Are there ... from the point of view of pro- ... , some of

It would be helpful for us to be very clear on what criteria we are going to use and to recognize that in a multidiscipline study of this kind no one criterion may be adequate for all the different approaches, and we may have to accept a number of them.

Now, Simms says aging is a multiplicity of lesions. Well, that is from one viewpoint—of the structures Somebody else says, from another viewpoint, that there is continued growth or changing functional capacities. We have a multiple set of criteria and now should begin to see whether, underneath those we can discover some concept of the dynamic processes that are operating in changing structure-functions (49), processes that have to be inferred.

Many findings, such as I have presented, point to the conception of a living organism as a dynamic configuration which persists only by a large and continuous expenditure of energy, if I may say so, just as crystal structures, the eternal rocks, are eternal only because of the enormous expenditure of energy within the crystal structure that makes up the lattice of the crystal.

These points are relevant to some of our discussion on the theoretical aspects of aging, and make clear the need for appraising and using all these contributions but not necessarily believing that we have to reduce them to one

Carlson Aging is not only a question of death, but of the gradual impairment of a great many functions, so that your abrupt end there, Dr *Lansing*, as to life span is largely theoretical

Aging is a gradual business, impairment of a great many factors, and I am tremendously impressed by the probability of using the topic of your presentation here, comparative physiology in all species, as an aid to the almost insoluble problem in man and mammals It is going to help us, I am quite sure. But let's not be too dogmatic about the apparent conclusion either from the ant or the bee Get the facts, but the absolute interpretation will come when we put them all together.

Simms· I would like to try to answer Dr Lansing's question Dr Lansing ... of curve than I ... my "log mortalit ... , I would like to translate what he was saying into my curves.

In Figure 51 I have drawn six curves corresponding to the hypothetical conditions outlined in curves 1 to 6 of Figure 50 presented by Dr Lansing I think, Dr Lansing, these curves will answer your ques-

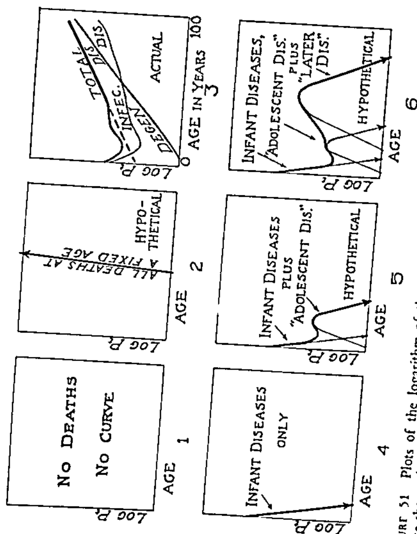


Figure 51 Plots of the logarithm of the probability of death (P_t) against age (t). These show what would happen in the hypothetical conditions of Dr. Lansing. Compare with Figure 50

tions as to (*a*) whether life span is limited by "multiple factors which debilitate the initial population at different points," or (*b*) whether they "culminate in extinction of the population at some arbitrary time"

To answer your first question, the hypothetical concept that temporary causes of death kill a portion of the population during a limited age period does not correspond with the facts.* The mortality from specific diseases (or from groups of diseases) does not have curves like those in my curves 5 and 6 of Figure 51, translated from your curves 5 and 6 of Figure 50. Instead, the curves resemble those in curve 3 of Figure 51.†

In answer to your second question, your case where everybody dies at a fixed age (your curve 2 of Figure 50 and my curve 2 of Figure 51) is, as you say, purely hypothetical. You call this a "built-in aging factor." I would be inclined to say that such a hypothetical situation would be the result of "identical susceptibility to certain disease(s) having a time lag." It has, as you can see, no resemblance to the actual termination of life span by the accentuated probability of death, represented in my curve 3, Figure 51 by the upper end of the actual mortality curve The increase in death rate with advancing age (as represented by the rise in this curve) is due, in part at least, to the effect of aging

Lansing· Dr Simms, what I really was asking was, is there a cause of death in addition to the degenerative diseases that operates to terminate life? And more specifically I asked, can ecological methods be used to characterize the presence or absence of such an inherent cause of death, which is what I think we mean by aging?

Hoskins Is there a universal lethal factor which every organism has?

Lansing Operating at different rates at different times

Simms Do you mean an aging factor?

Coudry A lethal factor

Lansing Is there such a thing as a cause of death? Is aging a cause of death as an inherent mechanism operating in the individual *in addition* to coronary disease, cancer, infectious diseases? Let me express it in another way If we eliminated these known causes of death—the cancers and strokes and coronaries, and polios, and what not, would we be left with an immortal population, or would that population still find that it had a finite existence?

Simms· I would say that aging is an *indirect* cause of death, not a

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[illegible] population

direct one. I conceive of the aging factor as playing a role in increasing the mortality rate. I don't picture aging as necessarily causing death directly, but rather that it accelerates the rate at which pathological lesions accumulate—thus producing a higher death rate.

I should like to finish this discussion by pointing out that the theory has been expressed that the increase in mortality rate with advancing age is due merely to the accumulation of primary lesions, which then lead to secondary lesions, and that there is more time for the accumulation of these primary lesions in the older individual. However, our recent data on the accumulation of lesions in the rat, which we have studied very carefully, indicate that this cannot be so, but that an aging factor must be involved.

For example, the primary lesions which do not contribute to the moribund state of the rat and, hence, do not influence the selection of rats for autopsy, follow a curve indicating an increased rate of accumulation of lesions as the rats grow older. If the accumulation of the primary lesions were purely random, influenced only by chance, then we would have a straight line going through the origin

That, I think, is clear evidence that the accumulation of the lesions cannot be accounted for on a random basis, but that an aging factor is involved To my mind, the influence of age on life span is as follows. First, the aging factor causes a progressively more rapid accumulation of lesions as the animal grows older, second, that results in a progressively greater mortality rate as we grow older, and that in turn limits the life span.

Shock: Doesn't the work that Dr. Lansing has done on changes in the blood vessels lead to the conclusion that there is something going on age-wise in a blood vessel that alters its susceptibility to the accumulation or arteriosclerotic lesions?

Lansing: Yes, I think I am one of those who agree that there are age-dependent changes occurring which condition the progress of the so-called degenerative lesion

Shock: Isn't that illustrated by Figures 50 and 51?

Lansing: Yes, indeed

Cowdry: I should like to propose another definition of aging. Aging is a change in a particular material, or individual, or cell, with time.

Now, I think that we try to be too specific We must realize that the planets age, and we age, and this change appears in many guises, but it is fundamentally a change with time in a particular body.

I believe that the factors that modify the speed and the character of these changes are tremendously numerous, but I still insist that it is change in the particular object with time

Carlson: We have got to think of certain specific diseases from

known infectious agents, and other factors such as a slowly developing arteriosclerosis, which the pathologist would not recognize as a specific lesion Now, in line with that, there is another factor which I think has to enter into our concept of aging If we consider this whole process of aging as shifts in modifications in cellular metabolism, then we think of the aging individual as one in which the powers of recuperation are slowed up. There is nothing that a pathologist can see in that, and yet the individual's ability to recover from the minor hazards, stresses, and fatigue factors of the day is different in a man at 70 from what it is in a man at 30 And yet such things as that, we have not yet been able to measure. So as a part of this cellular metabolism and its change from age 30 to age 70, one of the things is the capacity for recuperation and repair So that has to be considered also as a further modification of our idea that disease is not the only process that enters into aging.

Andrew: I think that it is rather difficult for the biologist, to speak of changes, fairly subtle changes, as lesions and as pathological changes But I believe that this may be partly a matter of definition, and I think that perhaps Dr Simms would be willing, say, to agree to include in the accumulation of lesions things like changes in the mitochondria that can be seen with the electronmicroscope, or changes in nucleoli, a vast number of things that you won't find in any textbook of pathology.

From time to time I have wondered whether we do not have here a somewhat analogous problem to that of the chicken and the egg which came first—natural death or reproduction? And it seems to me that reproduction indicates an escape from a necessary, natural cessation of life. Reproduction is s[illegible] species as such is able to [illegible] lesions, if we use that ter[illegible] such a different thing in the vast number of species that we have to have an extremely broad view of what constitutes pathology.

But I believe that the difference in viewpoints between Dr. Lansing and Dr Simms is probably more one of definition than it is one of actual opposition of views

Hoskins. Dr Gey, does your work throw any light on this problem? Has aging in tissue cultures been observed under optimal conditions?

Gey I do not believe so, but before I get into that, I want to say that I have been thinking of a situation in which as the individual gets older, his tissues may lose certain genetic components which are necessary to carry out specific functions In the case of thyroid tissue that undergoes malignancy, some groups of these malignant cells are incapable of incorporating iodine, meaning to me that this product of the originally normal cell has lost something from its genetic make-up

Now, why could not this be the case in aging: that the competence to carry on certain essential functions, not in a direction of malignancy, but in a direction of an aged cell, would be the loss of some necessary function reflected by loss of a genetic component? We need evidence on that point. How competent is the aged thyroid, cell for cell, or any other tissue which carries out a specific function?

Our work with tissue cultures has shown that we can get altered cells which are not malignant cells. This, then, it seems to me, points to loss of the wholeness of the cell in so far as its genetic composition is concerned. This is something that the pathologist could not see unless he could see the individual genes and test the cell lines for the presence of certain factors known to be there in full complement in an earlier period in the individual's life.

Cowdry: Do these altered cells which are not malignant carry on with the fixity in the same way that malignant cells do?

Gey: Yes, we see no difference in the permanence or stability of such change.

We have also seen, for example, the loss of transplantability of a cell that was formerly malignant, indicating that some factor essential for invasive properties on transplantation has been lost during a period of continuous cultivation.

Cowdry: We have no real reason to suppose that it is a sudden loss, though, have we?

Gey: We have no way of pinpointing these things and saying, "It happened here." We occasionally carry on transplantations in certain cell lines and find that the line no longer will produce a tumor. I am, therefore, greatly impressed with inherent changes that might occur in cells with time.

Restoration to a normal state would be, it seems to me, an impossibility. If something is lost, it is lost, and there is no chance to regain it, unless, of course, there were some pluripotential cells, i.e. some blastic cells, still left that could repopulate the tissue.

Simms: I have been using the word "lesions" in a broad sense, and I would accept your wording as to changes in tissue that involve loss of function as being a pathological change which would fall into the system as I was describing it.

In regard to Dr. Andrew's comments, the changes in the mitochondria might either predispose to lesions or changes in function, or might be involved in changes in function

Gey: It would seem to me that there are also other inherent factors One thinks of the Sequoia tree as living an awfully long time, perhaps the longest time on earth Is this an organism that is capable of producing more efficient antibiotics than any other that lives?

Simms: It is not the same tissue. In trees there is progressive formation of new tissue on the outside of the old dead tissue

Gey Of course, we don't know how much of a replacement there is in an organism All that we can say is that under conditions of elevated temperature, cells divide more rapidly than they do at reduced temperatures We can see this in all sorts of cell strains.

I don't know how one could proceed to find out how much of one's original thyroid was replaced at a given age period. Certainly, in the advancing years, this mechanism is slowed down, and one tends to accumulate deficiencies rather than getting a complete restoration from the more blastic cells which are responsible for providing new cells for replacement, following various injuries, or as in regenerative repair. I think this is a factor, too, that must be considered

Cowdry· Put it a little differently Going down to the viruses and assuming that they are degenerate microorganisms that have lost certain properties, there you have life and reproduction without aging, don't you? The virus multiplies and doesn't show a period of particle life, probably, in which that particle notably changes It looks to me as though the aging factor is reduced to a bare minimum and may not even be present

Carlson. Doctor, I am sorry you brought in the viruses, because we know so little about them

Cowdry: Oh, do we know much about aging? It seems to me we don't

Carlson We know more about the aging of the human than we do about the aging of the virus. Let's not confuse our problem by bringing in the virus

Gey· You will admit, will you not, that viruses can bore into the cell and disturb very much the genetic setup, and that in this way you might end up with a smaller complement of what is considered a whole cell than what you had before?

Carlson I wasn't arguing about the cell Dr Cowdry was speaking of the virus as an individual, if I understood him.

Cowdry: Of course, we can see it

Shock: When we try to infer the physiological characteristics of individual cells from data collected from an intact organism, we are always faced with the question of how many cells remain The effects we can measure may always be a combined result of changes in cell number and changes in cell function Some of the observed changes may be due to the accumulation of materials in the spaces between cells Hence, studies on different animal species, where the rates of cellular loss may be different, offers valuable information on the aging process.

Up to now, we do not have very much clear cut evidence that the functioning of a cell is related to its age. For example, the liver in an old rat will regenerate about as fast as in a young one (50). Thus, the capacity for growth and cell division has not been lost. More recent studies by Dr. Bourliere (51), in fact, have failed to substantiate earlier work indicating a slower rate of healing of superficial wounds in aged animals. We can at least conclude that for certain kinds of wounds, the healing rate is not substantially affected by age. Thus, we still need objective evidence that the physiological functions and capacities of individual cells change with age. As a physiologist, I believe there must be changes in cellular function with age which perhaps precede structural changes, but I think it is a problem which is yet unsolved.

Cowdry: I think that it is very important to view aging from a broad point of view, such as the point of view that Dr. Bourliere has expressed in dealing with different organisms, widely separated and having different life spans and different habitats, because I think that the statement, "The ways of nature are simple," is not true. I think the ways of nature are wonderful and complex, and I don't expect to see a simple explanation of aging, or one explanation. I expect to see multiple explanations, and it is work like Dr. Bourliere's that is going to bring forth the multiple explanations

Hoskins: I should like to have Dr Gross bring us some material he has prepared

Gross: Dr Hoskins, in his kind invitation to me to attend this meeting asked me to say something about the possible applications and limitations of electronmicroscopy to the field of gerontology. I should like to say something obvious right at the beginning, and that is that the electronmicroscope by itself probably is going to solve few problems alone. It will be used most effectively when combined with other instruments and other methodologies

The fact that the electronmicroscope is a relatively new and expensive instrument, does not make it all powerful and it is often a good thing to let it gather dust when the problem requires a different approach. We are primarily interested in the problem and not in the instrument

The problems that I am particularly interested in are those of the ultrastructure and reactivity of connective tissue components, and as I mentioned before, I came into it because I was interested in rheumatic disease. It soon became evident that it was very necessary to study normal structure and reactivity first I have done relatively little work on pathological material.

Actually, the work on the normal structure has led in a number of

xtremely interesting directions, and I find it more of an unwanted iversion to go off into pathology at this point, because perhaps some f these lines that we are surveying will produce some general prin-iples which will be applicable to connective tissue pathology.

However in keeping with what I was asked to do I will try to give ou some idea of how electronmicroscopy can be applied to a par-icular problem rather than emphasize results. This is best done by lemonstrating three different aspects of our study of the structure and eactivity of connective tissue.*

These, in general, are the connective tissue components (Table XI).

Connective tissue cannot be defined exclusively as an extracellular substance, since it consists of the mesenchymal cells, fibroblasts of numerous species, and the other cells, as well as the fibers, collagen, reticulin, and elastin. The ground substance is actually made up of a number of different materials: proteins, mucoproteins, mucopolysac-charides (only two of which are well characterized); there is soluble collagen present, there are plasma components; there are cell metabo-lites, and there is water and salts.

Wislocki: Are the mucoproteins clearly accepted as being distinct entities from mucopolysaccharides?

Gross: Yes, I believe there are a number of mucoproteins which are definite entities in so far as it is extremely difficult to disassociate the

TABLE XI

Connective Tissue Components

Cells — Fibroblasts — Numerous Species
Macrophages, Mast Cells
Fibers — Collagen, "Reticulin" Elastin
Ground Substance —
(Extracellular, Extrafibrillar Amorphous Matrix)
Proteins of Unknown Nature
Mucoproteins
Mucopolysaccharides
Soluble Collagen
Plasma Components
Cell Metabolites
Water and Salts

*This work was aided in part by grant number A90 (C3) from the National Heart Institute of the United States Public Health Service. This is publication 156 of the Robert W. Lovett Memorial Foundation for the Study of Crippling Disease, Harvard Medical School.

protein component from the carbohydrate moiety. They have be isolated primarily from glandular secretions and the blood.

Wislocki: Plenty of mucoproteins are products of glandular secr tions, particularly of glands which secrete externally, but I was askin particularly about the ground substance of connective tissue.

Gross: Yes, there is. One example is the mucoid of the cornea (52) It is also claimed that the hyaluronic acid of the synovial fluid i closely bound to protein, and therefore, this would be a stable muco protein complex.

Gey: There are no fatty substances present whatever?

Gross: There probably are. In the early work of Chittenden and Gies (53) on the composition of ground substance, it was found that there are tightly bound sterols of unknown origin present.

Hoskins: Do you regard the vitreous humor of the eye as a special case of this?

Gross: Yes. The vitreous humor is not only a clear, transparent amorphous gel, but it also contains fibers, as Matoltsy, Grignolo and I showed several years ago (54)

It is worth pointing out that the ground substance actually can be broken down into two general classes of materials: (*a*) the intrinsic components of the ground substance, which are the materials that come from the blood and pass to the cells, and (*b*) components which just traverse the ground substance of the connective tissue

Wislocki: Under "cells," in Table XI have you put down merely the ones that you are interested in, or is your list supposed to include all of the constant cellular components of connective tissue?

Gross: I don't think you can say it is a complete list These are the obvious ones There are probably other cells present. For example, the fixed reticuloendothelial cells might be considered connective tissue components, and cells that diapedese into the connective tissue are at one time connective tissue components, but the cells listed in Table XI are the obvious ones.

Wislocki: You have named mast cells but tissue eosinophils are equally ubiquitous and constant in connective tissue. Have you omitted eosinophils because they seem less interesting to you, histochemically speaking, than mast cells? They differ markedly from one another, each possessing distinctive cytological and histochemical characteristics.

Gross: I am sure there is a variety of cells present, and as people become more interested in special types, they will be more apt to be included in such a table. This is really just presented as a general outline of what the connective tissue components are, and it does not reflect their significance with regard to either quantity or function.

Cowdry: You have to include ...

chemical composition of connective tissue, nerve fibers to some extent, since they ramify through most of the connective tissue, don't you think?

Gross: Yes, but you have to make some useful, if arbitrary, limitations

Coudry: Yes, but if you are going to enumerate the kinds of substance, such as proteins and carbohydrates, and so forth, one would likely get another group of substances from nerve fibers, in small amounts

Gross: I am sure that is true in the case of all the tissues of the body. These are imbedded in the connective tissue and nerves are really imbedded in the extracellular matrix through which they pass, and these materials of the ground substance which I am defining here are extracellular. Now, as to the nerve, I believe it is generally considered by the cytologist that the axon substance is intracellular. The epineurium and perineurium should be considered as extracellular in origin. It is also possible that you might consider the myelin sheath as part of the connective tissue But that is where you have to decide where you are going to draw your arbitrary boundaries for utility's sake.

Andrew. I wondered whether the heading "numerous species" is intended to modify "fibroblasts," or "macrophages," or "cells."

Gross. The "numerous species" modifies the fibroblasts I think there is considerable good evidence Fischer in his book (55), and Parker (56) have shown that certain fibroblasts have specific metabolic patterns Certain of them produce bone, like the osteoblasts; certain of them produce cartilage, and others are involved purely in the production of collagen So there are very definite species of fibroblastic cells.

Wislocki: It is clear that there are such differences As a further instance

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with the

Gross. It is also worth pointing out that fibroblasts should not be classified on the basis of their morphology I think Paul Weiss (57) has shown that whether you get a great big diffuse stellate cell or whether you get a long thin cell with little cytoplasm is greatly dependent upon the nature of the environment itself, and that if these fibroblasts are grown in a fibrin clot of thin, closely packed fibers which are oriented by stretch, the fibroblasts will be long and thin, whereas if they are grown in a loose gel, the fibroblasts will be widely spread out with numerous processes So morphology is not a good index for classification

Wislocki. That is very clear As Warren Lewis pointed out years

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Wislocki. It is clear that there are such differences As a further instance, the fibroblasts of mucoid connective tissues, such as Wharton's jelly and the cock's comb, might be cited These appear to be associated with the production of sulfated mucopolysaccharides

Gross. It is also worth pointing out that fibroblasts should not be classified on the basis of their morphology I think Paul Weiss (57) has shown that whether you get a great big diffuse stellate cell or whether you get a long thin cell with little cytoplasm is greatly dependent upon the nature of the environment itself, and that if these fibroblasts are grown in a fibrin clot of thin, closely packed fibers which are oriented by stretch, the fibroblasts will be long and thin, whereas if they are grown in a loose gel, the fibroblasts will be widely spread out with numerous processes So morphology is not a good index for classification

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ago in his tissue cultures, if fibroblasts are grown against glass, they will in many cases spread out in the form of mesothelial sheets, in which the cells establish contact with one another in a mosaic-like epithelial pattern. This appears to be merely a case of mechanical adaptation of the fibroblasts to a particular spatial environment.

Gey: And conversely, one can say that the epithelium can be so disposed in space.

Gross: Now, the question of definition of the various components of connective tissue comes up. I will define the connective tissue the way I believe it to be most operational from the point of view of chemical reactivity

The collagen component, we believe, is best defined as a class of fibrous substances which give a characteristic wide angle X-ray diffraction pattern, and the members of which, particularly among the mammals, have a distinctive periodicity along the fibril axis of about 640 Angstrom units, or 64 millimicrons. Such fibers can be found in the animal kingdom, from mammals all the way down through the sponges.

Figure 52 shows the X-ray diffraction pattern characteristic of the collagen class of proteins. These fibrous proteins, from sponges on up through the egg capsule of the skate, which is secreted by cells, and including mammals, show this distinctive pattern. I won't go into the details of it. The various arcs and points you see here give the crystallographer considerable information concerning the molecular structure of this fiber (58)

An electronmicrograph of purified collagen fibrils from cowhide is shown in Figure 53 These have been produced simply by making a frozen section of a fresh tissue, washing it very thoroughly in water and sodium chloride to get rid of the amorphous ground sustance, and fragmenting it by gently teasing it with a needle, and allowing a bit of the suspension to settle on the specimen screen.

The mark at the lower left of the figure represents 1 micron, and you can see that the width of this large bundle of fibers is about 1 5 microns which means this bundle will be visible in the light microscope The individual fibrils will not, because they are only about 0 1 micron in diameter. You can detect them with a dark field microscope. The banded structure you see here, the characteristic 640 Angstrom period, the distance from one band to the next, is invisible in the light microscope. It is below the resolving power of the instrument. Notice that every one of these fibrils has the characteristic period, and the distribution curve of axial periodicity shows a relatively sharp peak at about 640 A. We use that as a fingerprint for the collagen fibril.

Having now a label which is intrinsic to this fibril, we feel that we



Figure 52 Wide angle X-ray diffraction pattern of collagen (Kangaroo tendon) Courtesy of Prof R S Bear

can make use of that label in studying the alterations that can take place in the fibril, both in experimentally controlled and perhaps sometime in disease processes Not only is the axial period characteristic of collagen but the detailed pattern of the intraperiod banding seems to be characteristic of most vertebrate collagen (59, 60)

One of the easiest types of collagen to examine is the collagen of skin

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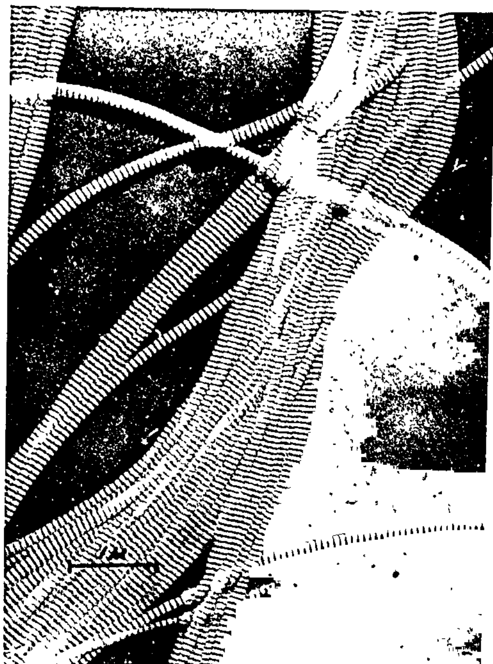


FIGURE 53 Electronmicrograph of purified cowhide collagen Mag 20000 x

In contrast, the collagen from young skin, prepared in exactly the same way as the old, is surrounded by a large amount of amorphous material in which the fibrils are imbedded.

Andrew: I think it might be pointed out that in very young animals

ie cellular population of the dermis is much heavier than in older nimals

Gross: Yes, that is true enough, but the intercellular matrix is also much more voluminous. Actually, the cells will be disrupted and pulled out of such solutions. I don't think that this material enmeshing these fibrils are cellular components, because it is possible to wash out most soluble proteins

Andrew: My point is that there is a striking difference that you see microscopically and that this amorphous material in the young skin might be cell products or food material going to cells, or something of that sort, which would make quite a difference in the intercellular material.

Gross: Oh, yes, there is no doubt about that, but this is sticky material which is probably not cellular, you can wash out the soluble proteins. But, you see, these fibrils are enmeshed in something that doesn't get washed off.

Lansing: Just as a matter of information, what is the difference between intercellular cement, described by Chambers, and ground substance?

Wislocki: The term intercellular cement is generally used in reference to the substance existing between epithelial cells as, for example, in the epidermis. Similarly, substance which is alleged to cement or glue endothelial cells together is very frequently referred to as intercellular cement On the other hand, the material that forms the ground substance of loose connective tissue, which Gross is talking about here, is very generally called ground substance

Lansing: But they may possibly be the same

Wislocki: They might conceivably be identical chemically No one knows

Gross: There is no knowledge as to the chemistry of the intercellular cement, and there is really very little knowledge of the chemistry of the ground substance—only in such things as tendon, skin, umbilical cord, and vitreous humour When you get into the parenchymatous tissues no one knows anything about it

If one treats such material with trypsin, the ground substance is destroyed and one sees that the characteristic collagen fibrils are now fairly clean They are enmeshed in material which can be destroyed by trypsin I should say hyaluronidase does not do quite as good a job on this as does trypsin

One of the problems that the electronmicroscope can be used for is to study changes, in the same way as histological methods, with time, for instance, in the aging process I should like to show the highlights of one such study that we have been making on the aging of collagen

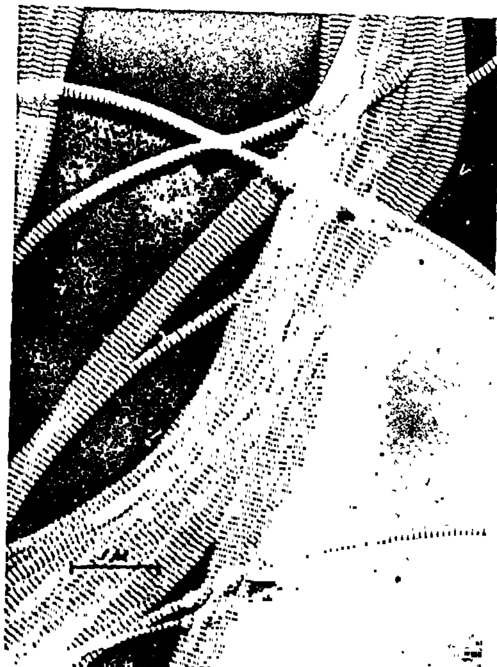


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fibrils in the skin of the rat, primarily for the purpose of learning something about fibrogenesis (62). Also, we thought we might be able to get a little information about the difference between reticulin and collagen.

In the newborn rat, if one stains by Bielchowsky's method, or any of the silver methods, practically 90 per cent of the skin is seen to consist of argyrophilic fibers, and as far as I can tell from the literature, the only one criterion of reticulin that all people will agree on is the argyrophilia. Some people claim reticulin contains more or less sulphur; others say it is not birefringent; there is very little agreement as to whether it is soluble in certain enzymes and as to whether it swells or doesn't swell in acid. The problem is complex because of the difficulty of separating these reticulin fibrils from the rest of the material they are enmeshed in.

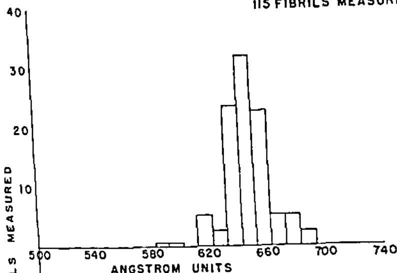
It does not make any difference whether one is willing to call these argyrophilic fibrils reticulin or not. In the newborn rat skin they give characteristic X-ray diffraction pattern of collagen and they also have the characteristic axial periodicity of collagen in the electronmicroscope (Figure 54). The materials for these experiments were prepared by freeze sectioning the full thickness of the fresh corium of the animal's flank and releasing and cleaning the fibrils by incubation in crystallized trypsin and washing the suspended fibrils in water in the high speed centrifuge. A drop of the resuspended fibrils was deposited on the supporting fibrin of the specimen grid, drained and lightly shadowed with chromium. Others were stained with phosphotungstic acid.

Figure 55 shows a bundle of fibrils from newborn rat skin for comparison with a bundle from a 90 day old rat (Figure 56). The magnifications are just about the same. The difference is that the fibrils from the young skin are very much thinner than those from old skin, and one sees why a bundle of reticular fibrils will seem to branch, not that the individual fibrils branch. The individual fibrils are very uniform in width. Branching is not a characteristic of collagen fibrils, and it is not a characteristic of the "reticulin" fibrils.

Cowdry: The collagen fibers are directly continuous with the reticular. If one could get those under the same magnification also, it would be very interesting.

Wislocki: Although the fibrils don't actually branch, nevertheless individual fibrils, as they age, do appear to grow both in length and width, do they not?

Gross: That is a moot point, and one that has come up during the course of this study. The characteristic of this newborn rat skin collagen, or reticulin, is that the distribution of fibril widths is extremely



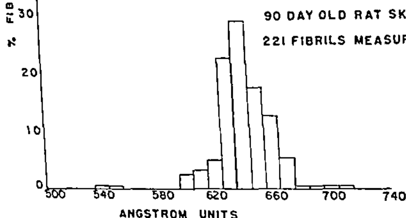


Figure 54 Distribution curve of axial periodicity in fibrils from 2-day and 90 day rat skin

sharp There is very little dispersion in width between the ages of 2 to 12 days In the adult rat, the average fibril runs somewhere between 1000 and 2000 Angstrom units wide.

Figure 57 is a picture of the collagen from the skin of a 28-day-old rat

Carlson Is that the same magnification?



FIGURE 55 (above). Electronmicrograph of "reticulin" fibrils from a 3-day-old rat skin. Mag. 24200 x

FIGURE 56 (below). Electronmicrograph of collagen fibrils from 90-day-old rat skin. Mag. 24400 x.



FIGURE 57. Electronmicrograph of collagen fibrils from 28-day-old rat skin Note marked variation in fibril width Mag. 34000 x

Gross Somewhat higher From about 12 days on, the histological picture of the newborn rat skin is one of decreasing argyrophilia and increasing collagen-type staining You observe in the electronmicrographs of such skin an increasing dispersion of fibril widths, and here one see fibrils that are apparently adult in diameter and mixed in with fibrils that are typical of the newborn skin, of about 300 to 500 Å in diameter In no instance among hundreds of such preparations that I

have looked at, have I ever seen two of these thin fibrils come together to make one big one, and it is my impression that what is happening is that the fibrils that are first laid down by the animal stay there and don't change. Then as the animal grows, the new fibrils that are laid down are larger in diameter until finally in the adult animal there are so many large collagen fibrils that they statistically overwhelm the smaller ones. As Nageotte and Guyon (63) showed, if you inject a little saline into the connective tissue to form a bullus and stain it, you can see that there are plenty of reticulin fibrils.

Wislocki: That leads to the very interesting question of the origin of the fibrils, or the fibers, both in the younger and older stages. You are maintaining that in the older ages they are larger at the time of their formation Moreover, your concept differs from the recent observations of Keith Porter (64) which indicate that collagenous fibrils are formed through the activity of fibroblasts. He describes the fibrils as forming in the cytoplasm and being ejected at the surface of the cells.

Gross: First I can't entirely agree with Keith Porter's observations as to the formation of fibers. I think there are certain indeterminates in this type of work that you just can't resolve by the methods in use. For example, just because fibrils appear to be close to cells does not mean they are formed there. In the drying of the preparation, these fibrils then are plastered on the cell surfaces. I don't think Porter has ever answered this objection satisfactorily.

Gey: Nor have they found any continuity between fiber and a portion of the cells

Gross: He claims he has

Wislocki: He claims he has, and shows pictures in which you see them very definitely inside the cells.

Gey: I have seen them and am not quite convinced

Gross: You remember how I was fooled on the elastin story. Electron micrographs showing one thing deriving from another should be considered guilty or at least suspect until proved innocent.

Wislocki: The question of the mode of fiber formation is a moot but important one.

Gross: A series of distribution curves of fibril width at various ages is shown in Figure 58 Each curve represents a frequency distribution of the widths of about 100 to 200 fibrils They were selected from a series which included 17 animals and are representative.

The curve for the 2- to 3-day-old rat skin shows the average fibril width to be somewhere around 400 Angstrom units. The dispersion is quite narrow. When one goes to the 18-day-old animal, the mean is increased, and the dispersion is increased. Similarly, with 28 days

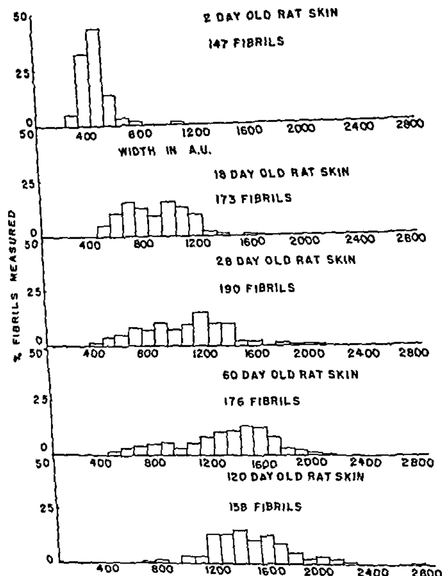


FIGURE 58 Frequency distribution curves of fibril widths from the skins of five rats of various ages selected from a group of 17 animals Note increase in mean width and dispersion

the dispersion is further increased, and the average width is again shifted Beyond 60 days the dispersion is quite large There are still some thin ones in every case, but the average width has increased considerably

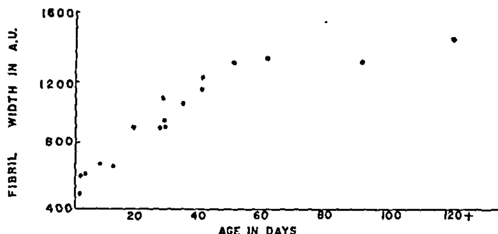


FIGURE 59 Change in mean fibril width with age of rat

Figure 59 is a plot of the mean fibril width for a series of animals versus age in days. You can see that the change in fibril width is a very uniform one for biological material and seems to level off at about 60 days.

Cowdry: Is there a regional difference in the animal?

Gross: Yes, there is, and we have checked this For example, one thing that worried me was whether fibrils from the top of the corium would have the same width distribution as those from the bottom. This was checked by examining a fragmented frozen section from the top and one from the bottom. The small difference found is not a factor in these studies.

Simms: Do you know what happened after 120 days of age?

Gross: Yes We have examined the fibrils of several animals 2 years of age, and found that they are very little different from what we found at 120 days

McCay: What happens before that in the embryo?

Gross: We have made some preparations from embryo skin It is rather difficult to get uniform dispersions of fibrils because of the gelatinous matrix, which is not very readily destroyed by trypsin. The fibrils found were smaller and had the typical collagen axial period of about 640 Å. They go down to around 200 or 300 Å in width in an embryo about halfway to term. We have not done enough really to be able to say very much about it.

One of the indeterminacies in this type of work is the fragmentation procedure. If groups of fibrils of a particular width do not fragment as readily as those of another width, then we will be selecting and not obtaining random samples. It is not easy to interpret what this selection factor is. All that can be said is that with this procedure a particular

pattern is observed. I believe that the recent evolution of the sectioning technique is going to answer this problem a little more exactly than the fragmentation method which was the only one available when this work was done.

One obvious thing to check was the differences between "reticulin" and collagen and the nature of argyrophilia. Some of the newborn and adult rat skin was stained by the standard Bielchowsky procedure, gently fragmented and examined with the electronmicroscope.

Figure 60 shows "reticulin" from a newborn rat stained with silver. The process appears to be the deposition of silver particles. Using the dark field microscope, Nageotte (63) suggested that this might be the case

In contrast, Figure 61 shows collagen from the skin of a 90-day-old rat stained with silver Notice that the distribution of the particles *seems* to be different The particles are more irregular.

The fixation with formalin and the various procedures that one goes through with the silver staining methods tends to disrupt the structure, but actually we have not studied the silver process enough to say, "This is why collagen stains brown and reticulin black."

Another type of problem of a more dynamic sort, which is amenable to study with the electronmicroscope, is fibrogenesis of collagen. Nageotte (65) and several others found that some types of collagen such as that from rat tail or the swim bladder of the carp, will dissolve in dilute acid quite readily, giving a highly viscous solution This can be filtered through fine glass filters, and examination of these clear filtrates of collagen shows no fibrils present.

Figure 62 shows such a filtrate, an acid solution of collagen from rat tail tendon Notice the extremely fine filaments in the background as shown in a representative field One does not find any formed fibers

Nageotte observed that the addition of NaCl to such a solution produces a fibrous precipitate (65) He believed that perhaps collagen fibrils might be laid down extracellularly by some similar mechanism

Schmitt, Hall, and Jakus (66) studied this phenomenon in 1943 with the electronmicroscope, and they found evidence of axial periodicity in these precipitated fibrils

For the last five years we have been studying this process of fibril formation in collaboration with F O Schmitt and more recently J. H Highberger, and I shall go into just one aspect of it

Addition of 10 volumes of 1 per cent NaCl produces a fibrous precipitate which consists of fibrils with the same structure as the native collagen (Figure 63) We are able to produce a highly structured fibril from a solution of building blocks, presumably without the use of

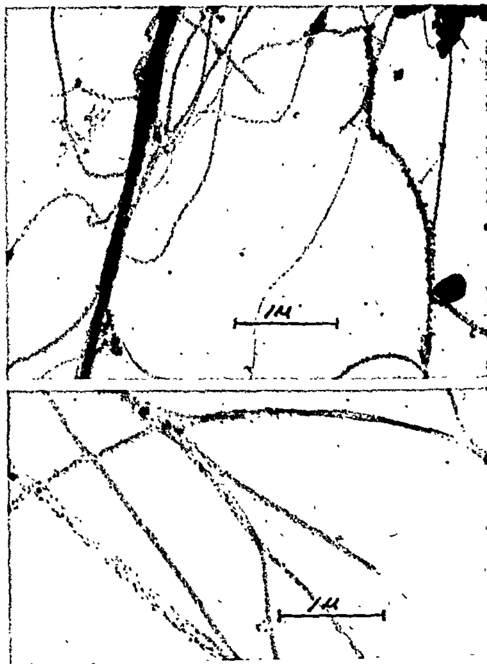


FIGURE 60 (above) Electronmicrograph of fibrils from section of 2-day-old rat skin silver stained by the Bielschowsky method and gently fragmented Mag. 23800 x

FIGURE 61 (below) Same as Figure 60 but prepared from 90-day-old rat skin There is a loss of axial periodicity Mag 23800 x.





FIGURE 62 (above) Electronmicrograph of filaments in a clear acetic acid solution of ichthyacol Mag 48000 x

FIGURE 63 (below) Electronmicrograph of collagen fibrils precipitated by 1% NaCl from solution shown in Figure 62 Mag 28000 x

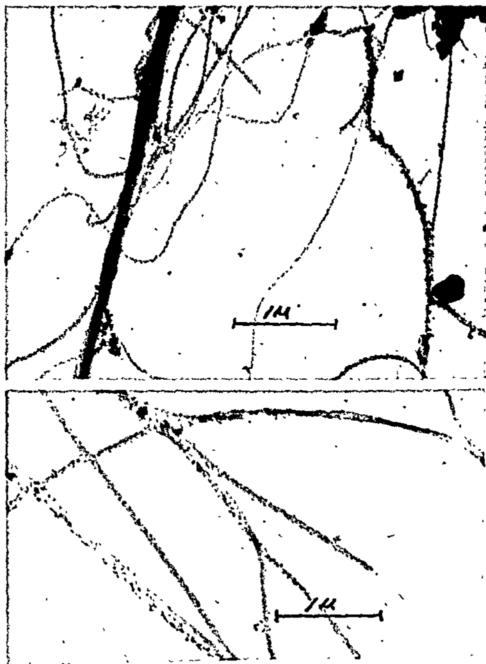


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FIGURE 61 (below). Same as Figure 60 but prepared from 90-day-old rat skin. There is a loss of axial periodicity. Mag 23800 x.

enzymes, just by a simple modification of physical and chemical environment

Gey: Would you surmise that a similar process goes on in the body?

Gross: That would be a big jump. I don't know But it shows that it is possible for a highly structured fibril, very much like that which is present in the body, to be formed through the interaction of macromolecules under the influence of physical chemical stimuli.

Cowdry: You could probably get it with a very much reduced concentration of salt, couldn't you?

Gross: Yes, you can go down below 1 per cent. Even the detailed intraperiod fine structure can be exactly reproduced

Carlson: Is there any way of measuring the relative elasticity of the fibers found in the tissues or made as you made them here?

Gross: Not very well, Dr. Carlson. These things will stretch in the electron beam when the collodion film on which they are supported breaks (*60, 66*). But no one has ever shown significant extensibility in a macroscopic bit of collagen. What this means in terms of molecular structure, we don't quite know.

If one increases the salt concentration to 2 per cent, the periodicity observed in the precipitated fibrils is reduced to about 220 Å instead of 640 It is interesting that Keith Porter has found in his tissue cultures fibrils having a periodicity of about 250 Å

One can find fibrils with periods of 220 Å in animals of various ages: newborn, old ones They are rare, and we think that it might be a matter of technique They might also result from the filamentous elements in these fibrils being in poor lateral register, so that the 640 Å period does not stand out sharply. In the upper part of Figure 63, for example, in some of these fibrils you can just see the periodicity beginning to peek out—the main periodicity of 640 Å It is worth noting th[illegible] a 220 Å period when sh[illegible] when stained with phosp[illegible] metal shadowing can occasionally be quite deceptive

When one increases the salt concentration to 3 per cent, one gets a

FIGURE 65 (middle) Fibrils from same preparation after 1 hour digestion in collagenase from Cl Histolyticum Note tapering ends Mag 13300 x Reprinted, by permission, from Gross, J An evaluation of structural and chemical changes in connective tissue components *Ann N Y Acad Sc* 56, 674 (1953)

FIGURE 66 (below) Same preparation after 4 hours digestion [illegible] tapering and diminution of fibril width with preservation of a[illegible] Mag 17500 x Reprinted, by permission, from Gross, J. An evaluation of structural and chemical changes in connective tissue components *Ann N Y Acad Sc* 56, 674 (1953)

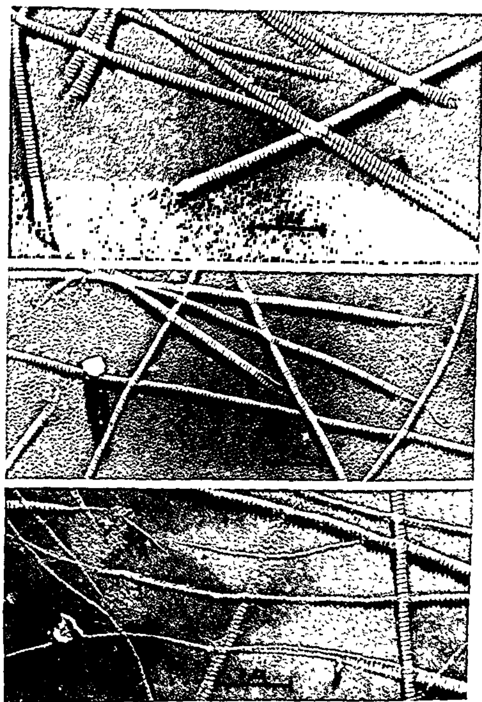
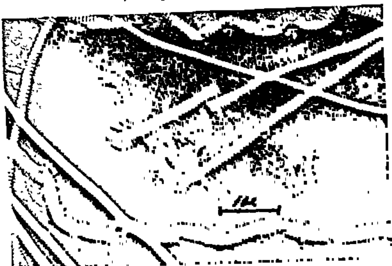


FIGURE 64 (above) Collagen fibrils from purified cowhide. Note squarely cut fibril ends Mag. 16100 x. Reprinted, by permission, from Gross, J · An evaluation of structural and chemical changes in connective tissue components. *Ann N. Y. Acad Sc.* 56, 674 (1953)



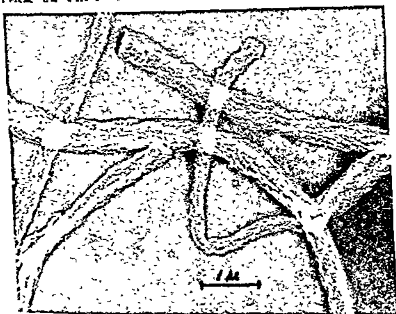


FIGURE 67 (above) Purified cowhide collagen fibrils treated with 0 1 *N* HCl for 1 minute Mag 13300 x Reprinted, by permission, from Gross, J An evaluation of structural and chemical changes in connective tissue components. *Ann N Y Acad Sc* 56, 674 *(1953)*

FIGURE 68 (below) Same preparation as shown in Figure 27 after 3 hours in HCl Mag 13000 x Reprinted, by permission, from Gross, J An evaluation of structural and chemical changes in connective tissue components *Ann N Y Acad Sc* 56, 674 *(1953)*

fibrous precipitate with no periods at all. The concentration of salt can modify profoundly the structure of precipitated collagen.

We have done much work on the interaction of glycoproteins and ATP with collagen, and have found some rather remarkable structure formation, which I just don't have the time to go into at this point. There are other agents besides salt which can organize dissolved collagen into fibers and "crystals" (67, 68, 69, 70).

Another type of work that is possible with the electronmicroscope is the study of model systems of abnormality: in other words, starting with a pure preparation of collagen fibrils which are clean well identified and then treating them with such things as acids, alkali, swelling agents, and various enzymes to try to get some base line for the reactivity of collagen itself under normal conditions (71).

Figure 64 shows fibrils from steer hide which have been purified by washing with salt and phosphate solution. The ends are cut off fairly square by the freezing microtome in the process of fragmentation. These come from a suspension of fibrils in water. A small mass of such fibrils is then placed in dilute hydrochloric acid, and then after a minute, a drop is deposited on the specimen film. One sees some obvious alterations in the fibrils.

After short treatment with acid, you can observe that there is fragmentation, fraying, twisting, some loss of structure, but a good number are still normal (Figure 67).

After three hours in hydrochloric acid, these collagen fibrils have now lost most of their structure (Figure 68). If one found such a fibril in which the axial period is completely obliterated in a lesion, such as a rheumatic fever nodule, one could not say that particular fibril was collagen, because it would have nothing to identify it. It is a fibril of unknown derivation However, as long as a fibril shows some evidence of the characteristic fingerprint or periodicity characteristic of collagen, one can say that it is an abnormal collagen fibril.

Cowdry: Could you restore the periodicity of those fibrils (Figure 68) by treating with salt?

Gross: You can with certain types of tissue but I am not sure about skin collagen.

It has been said that collagen is not profoundly affected by dilute alkali in the cold. However, if one treats purified collagen with dilute sodium hydroxide, one observes marked changes after one hour. The fibrils are fragmented; they are frayed; there is a lot of amorphous material present (71).

Trypsin, we found some time ago does not affect the collagen structure.

Pepsin, however, does cause marked destruction. The first effect you

I am trying to show is the application of a technique to the study of tissue changes, and I think the application of such studies to aging will appear in the future. Two obvious relationships are to wound healing and to sclerosis. I think that any information that is obtained as to the nature of the deposition of collagen will eventually give us some leads about processes in wound healing, embryogenesis, and perhaps in pathological sclerosis.

Coudry: Have you taken any fibers from arteriosclerosis?

Gross: Well, I have looked at arteriosclerotic aortae and have found lots of normal collagen and lots of amorphous-looking thin fibers. The amorphous fibers have no label on them, so I can't tell what they are. But they do look somewhat different from the fibers of the aorta in newborn animals.

Coudry: Suppose you used, instead of salt, potassium?

Gross: Potassium works like sodium Calcium does not reconstitute collagen like this.

Hoskins: Gentlemen, we have a scant half-hour left The thought that I had at the beginning of the period was that we might favor ourselves with a little more discussion of Dr. Bourliere's paper and get his comments on the things that have been said in the meanwhile. Dr Bourliere, do you want to take the floor and give a few free associations?

Bourliere. I just wanted to ask a question in connection with this matter of hibernation and what is often called "state of cellular and tissular quiescence" At the present time we know very little about the biochemistry of hibernation and I should like very much to ask Dr Wislocki's opinion on this problem Hibernation in any way seems to have a very favorable effect in slowing down the aging processes and its study is of fundamental importance from our gerontological point of view. Dr Wislocki, how do you think that hibernation acts in slowing down the metabolic processes?

Wislocki I know very little about that The two investigators in my laboratory to whom I referred yesterday found that hibernation inhibited the growth of cancer transplants until hibernation stopped, when the transplants immediately resumed their growth. People may have cultivated cells from hibernating animals in tissue cultures, but I am not actually familiar with any such reports

Gey: Undoubtedly some work of this sort has been done I can't cite any specific instances now. I recall an early interest in the role of the interscapular gland when I first came into the Anatomy Department and whether one might extract from this gland, if it is important, a factor which might render cells more dormant than they would be under other circumstances I know of no particular experiments in this direction.

see after treatment with pepsin, at pH 2 or 3, is marked swelling, the type of swelling that you see with just the acid alone. However, after several hours there is evidence of enzymatic activity. One sees fibrils that look rather reminiscent of the effect of the dilute acid. Pepsin operates in an acid range, so it is hard to dissociate the effects. However, large masses of amorphous material soon appear, after about an hour. And after four hours fibrils are gone completely, and all that is left are masses of amorphous junk. After 24 hours, even these things are gone, so there is complete dissolution (71).

One enzyme that we have been very much interested in is collagenase, and Dr. MacLennan from Columbia has provided us with some active bacterial collagenase, which we know destroys native collagen. First, we were interested to see how this enzyme operates on the collagen fibril (71) and secondly, we wanted to study the breakdown products to see if we could get some information on the components of the fibril.

You remember what the normal collagen looked like in Figure 64. Figure 65 shows what happens when these collagen fibrils attacked by the enzyme after one hour, and you notice that the ends are markedly tapered There is certainly loss of substance in these fibrils, although the periodicity has not been disturbed at all. Figure 66 shows the marked tapering that occurs after several hours' treatment with collagenase but the fine structure has not been altered. Material has been peeled off the surface

What we have tried to do here is to begin to establish some range of reactivity for normal collagen, with the idea of going step-wise to the study of more complicated tissue complexes armed with the knowledge derived from examination of the range of reactivity of purified constituents.

Cowdry: Does the persistence of the periodicity indicate that the periodicity is due to some substance other than that attacked by the enzyme?

Gross: I am not sure of the answer, Dr. Cowdry. We know that there are carbohydrates in the collagen fibril, in purified collagen. We have extracted several different sugars, characterized by paper chromatography

Chow: What happens to the normal fibers if you increase the sodium chloride concentration?

Gross: Nothing. There is no obvious effect on an intact fibril, at least in the electronmicroscope, with increase in salt concentration.

Carlson: Will you tell us now briefly the bearing of your work to date on aging?

Gross: Frankly, Dr. Carlson, I don't feel that at the moment I can relate this particular work to aging nor have I tried hard to do so All

hamsters are refractory to the action of colchicine, so that it proved impossible to contrast the rate of mitosis in its tissues in the hibernating and normal states.

Bourliere: Yes, in my hibernating snakes one gets a very great decrease in the activity of the heart There is roughly one heart beat per five minutes.

Wislocki: I would guess that if one were to culture tissues from a hibernating hamster, they might not actually divide and grow until the tissue culture was brought to ordinary body temperature. As long as it was kept at the hibernating temperature, I would rather doubt that the cells would grow.

Gey: Wouldn't it be necessary to provide the specific hibernating host fluid in your tissue culture and not use something totally different from the host?

Incidentally, we have Chinese hamster cultures running now, but not because we are interested in the hibernation aspect. I don't even know that these small animals do hibernate. It may be that they are like the Serbian hamster Our interest in them is only in connection with the very small number of chromosomes that they have

Carlson: In these small animals that hibernate for one or two months, is there any development of anemia at the end of that hibernation?

Bourliere: I do not think so

Hisaw: There is a development of anemia reported to occur in frogs during hibernation, so much so that when they come out of hibernation, hemopoiesis takes place in the spleen as well as bone.

Carlson How about the mammal?

Bourliere. I do not have the literature in mind, but my feeling is that there is no change

Hisaw: But in connection with the ability of the cells to divide, I might say that the hibernating 13-lined ground squirrel can respond to estrogen while it is in hibernation and produce normal growth of the uterus and the vaginal epithelium

Wislocki: The only experiments done upon tissue growth or regeneration in hamsters in my laboratory have been those on homologous cancer transplants alluded to several times previously Hibernation does not kill the transplants They remain dormant or latent during the period of hibernation, and then, when the animal comes out of hibernation, the grafts take and cancerous lesions develop

Andreu One peculiar histologic change that occurs in hibernation is outlined in an article [illegible]

the frog including [illegible]

We know, on the basis of experiments where we have withdrawn protein and lowered temperature, that we have greatly reduced the requirements of cells for over-all nutrients that one might add to a tissue culture. By following the mitotic activity of a tissue in such situations, one can see that the over-all activity of the tissue is cut down, using the prolongation of the inter-mitotic interval as one yardstick.

On the basis of treatment schedule, one might say that an economy of the order of fifteen times could be effected by changing the temperature from 37 to 28° C. To give you a specific example of some work we published some years ago, a strain of fibroblasts could be carried on a sort of "indefinite" basis by treating once every two months and transferring once every four months, whereas in a rapidly growing culture, at 37° C.—and this is still not isothermal for the host—the treatment schedule imposed by the conditions of cultivation would be of the order of two times a week.

Bourliere: Is there any information about the enzymatic activity in cells or tissues kept in frozen or in quiescent condition?

A lot of work has been done on the survival of fowl and cattle spermatozoa "frozen" at –79° C., and even isolated tissues of higher vertebrates seem to be able, in very definite conditions, to withstand very low temperatures for a long time

Gey: From Dr Polge's laboratory, I take it?

Bourliere: Yes, from Dr. Polge's (72, 73) laboratory in England, and other laboratories too. Quite recently Gonzalès and Luyet (74) reported, for instance, the survival of heart cells of chick embryo after freezing in liquid nitrogen (–195° C) for four weeks.

Hisaw: Just recently they have reported that they have been able to maintain spermatozoa that have been frozen in that way then brought back to a temperature of about –20° C and kept in the deep freeze for a very long time. Then they have been used for artificial insemination in cattle, with success

Cowdry: I think it is clear that the suddenness of the freezing and the absence of crystals makes all the difference and that if you get a state bordering on complete inactivity without disruption, the cell will last almost indefinitely

Wislocki: Some of these questions, of course, can be tested. In hibernating hamsters, activity, respiration, heart rate, body temperature and other functions are very much reduced On the other hand, cellular activity during the hibernating state has not been extensively investigated. The rate and character of experimental wound healing during hibernation might yield interesting information. Also, the study of the rate of multiplication of cells from hibernating hamsters might be rewarding. In *Science* recently it was reported that the tissues of

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nation, actually new neurones are formed from neuroblastic cells that are present there in the plexus.

Carlson: Is that true in both young and old frogs?

Andrew: That was in adult frogs. I don't know how old they were.

McCay: As a result of Dr. Bourliere's discussion, I think this group could very well stress the importance of the turtle for studies on aging. Far more attention could well be given to the physiology and life of the turtle. It is one animal that we could get by the thousands, if we wanted to, and have material for several generations to work on.

Simms: What is the life span of that species of turtle?

McCay: I haven't any idea.

Bourliere: Your turtle fauna is much richer than ours, and you have, in this country, excellent opportunities to work on their physiology and biochemistry.

Hisaw: Since in the closing moments we are talking about turtles, I should like to bring this to your attention: if you want to work on turtles, why not work on the Ozark terrapin? There are great numbers of them. They are on dry land. They are nice clean animals. You can keep them in captivity easily enough. People have cut their initials on them for generations. Evidently they live for a very long while, but nobody knows how long.

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A STUDENT OF AGING LOOKS AT THE MACY FOUNDATION FOR SEVENTEEN YEARS *

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THE INTEREST OF the Josiah Macy, Jr. Foundation in problems of aging developed from the publication of the symposium upon arteriosclerosis, entitled *Arteriosclerosis: A Survey of the Problem.* This was edited by an eminent histologist who was also a man of much vision, Dr E. V. Cowdry of St Louis, and appeared in 1933 In this work substantial attention was devoted to the possible role of cholesterol in the development of arteriosclerosis with an excellent historical summary in this field starting with early Russian research which antedated World War I

Members of the Board of the Macy Foundation during the period from 1930 to 1936 must have felt that the study of cholesterol was one of the most promising fields of attack upon the problem of sclerosis because during this period grants of about $160,000, nearly one-fourth of the total sum for research grants, were made for research upon cholesterol, arteriosclerosis and aging About $68,000 of these grants were aimed specifically toward studies of cholesterol. Something was learned about the metabolism of cholesterol, but the resulting advances in knowledge of human arteriosclerosis were quite meagre. During this same period starting in 1930, only about two per cent of the funds invested in research grants was allotted to basic studies of aging. The number of projects supported in each field was the following. cholesterol, fifteen, arteriosclerosis, thirty, and aging, six The average annual grant for the study of aging amounted to $2,374, while the average for the study of cholesterol was $4,527.

During the period of 1937-1940 the interests of the Foundation shifted substantially because only twelve per cent of the research budget was invested in the combined fields of cholesterol, arteriosclerosis, and aging. In this period the funds made available for basic studies in aging amounted to more than $57,000, although the combined funds for the study of aging, cholesterol, and arteriosclerosis amounted to only twelve per cent of the funds granted

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*This statement was prepared in response to a request by the Conference members

69. HIGHBERGER, J. H., GROSS, J., and SCHMITT, F. O.: The interaction of mucoprotein with soluble collagen: an electron microscope study. *Proc. Nat. Acad. Sc.* **37,** *286 (1951).*
70. SCHMITT, F. O., GROSS, J., and HIGHBERGER, J. H.: A new particle type in certain connective tissue extracts. *Proc. Nat. Acad. Sc.* **39,** *459 (1953).*
71. GROSS, J.: An evaluation of structural and chemical changes in connective tissue. *Ann. New York Acad. Sc.* **56,** *674 (1953).*
72. POLGE, C.: Functional survival of fowl spermatozoa after freezing at —79° C. *Nature* **167,** *949 (1951).*
73. POLGE, C., and ROWSON, L. E. A.: Fertilizing capacity of bull spermatozoa after freezing at —79° C. *Nature* **169,** *626 (1952).*
74. GONZALÈS, F., and LUYET, B.: Survie de cellules du coeur d'embryon de poulet après un séjour de quatre semaines dans l'azote liquide. *Compt. rend. Acad. d. sc.* **234,** *2651 (1952).*

pessimism among the biologists as the edition changed from the first to the third. This is undoubtedly a reflection due to the fact that about one-half of the authors of the original edition had passed away before the third edition appeared, and some of the youthful enthusiasm of the younger writers had been tempered by twelve years of life and the experiences of a major war with research directed toward making warriors of the young rather than improving the health of the older people.

After the pioneer efforts of the Macy Foundation in getting a small group of specialists together at Cape Cod, there were fourteen more annual meetings carefully organized by the Foundation. About ten of the original members were usually present with about fifteen others, part of whom had joined the group shortly before the publication of the second edition Some of these "second edition" members such as E J Stieglitz and R A Moore added much in both vision and virility to the annual meetings Likewise, new life came into the group as enthusiastic critics such as Nathan Shock, Henry Simms, and E Shorr joined actively in the discussions

As we examine trends in the field of gerontology about 1942, we discover fields of interest that had reacted to stimulation from the activities of the Macy Foundation In the first place, interest in possible relationships between cholesterol in the diet and arteriosclerosis had continued although even today there is still much question about the importance of relationships between the sterols consumed in the diet and sclerotic changes. Even proving the questionable existence of such realtionships is valuable, because newer working hypotheses have to be created and interest in this important field is thus sustained.

As noted by Cowdry in the preface to the second edition of *Problems of Ageing*, a number of conferences were organized just prior to 1942 to consider the economic, social, and medical problems of our aging population Some of these meetings would undoubtedly have occurred even if there had never been a Macy Foundation because the depression had created a substantial increase in interest in providing pensions and relief for older people Thus the United States tardily followed many European countries in attempting to establish a pension system rather than public relief as a means of supporting millions of older people who had been unable to provide for some financial security for their later years This development increased interest in problems of pensions and retirement Likewise, the ever increasing burden of the aged in public institutions such as state mental hospitals invariably stimulated interest of psychiatrists, clinicians, tax payers and public officials in problems of older people

However, the meetings under the stimulus of the Macy Foundation set a pattern for discussions by small groups of specialists. The pub-

However, during this period the Foundation assembled a group of researchers who were then working directly upon problems of aging or who were engaged in research in the basic science fields which afforded possibilities of contributing to the knowledge of aging. Dr. E. V. Cowdry was again chosen to edit the first edition of *Problems of Ageing, Biological and Medical Aspects.* In order to acquaint the authors of the book with each other, since they had to come from very diversified fields, a unique method was devised by L. K. Frank, namely, to assemble the group for a few days at one of the older hotels in the village of Falmouth on Cape Cod.

Here the group spent several days together, talking over plans mornings and evenings and at meal times In the afternoon the group swam and talked some more

Among the twenty men photographed at this conference on June 26, 1937 were A. J. Carlson, A. E Cohn, Wm Crocker, E V. Cowdry, L. I. Dublin, E T Engle, L. K. Frank, J. S. Friedenwald, W. S. Hunter, H. S. Jennings, E B. Krumbaar, Wm. deB MacNider, C. M. McCay, E D Merrill, W. R Miles, J. R. Oliver, T. W. Todd, F. Fremont-Smith, and C. Wissler

Among this group were a few such as Dr Dublin, who, because of his association with an insurance company, had for a long time been concerned with the aging of man. Others such as Dr Todd had been led into the problem in the course of studying the growth of the skeletons of children and later changes in the adult Still others were interested in special organs Among these researchers were some, however, whose interests might seem far afield from the aging of man, such as Crocker and Merrill who were famous plant scientists However, no one could say that the plant cell might not unravel the basic problem of aging so the basic plant scientists deserved recognition in this first attempt to compile a book containing the known knowledge in the field.

An interesting comparison of the three editions of *Problems of Ageing* which appeared in 1939, 1942, and 1952 has been presented by T S Gardner In Gardner's introduction he describes these editions as "the major contributions to the field ever published and have served as unifying forces in thinking and working in aging research" [*J. Gerontol. 8:349 (1953)*]

This work proved useful to the small group of scientists who were concerned with research in the field Parts of it were also read by a small number of older people who hoped to find some knowledge for personal application. Possibly the best service of this book was the bringing together of scientists concerned with aging. Between the second edition in 1942 and the last in 1952, the number of writers increased from thirty-six to forty-seven. Gardner noted the growing

organized the excellent conference during May of 1941, which later published its papers in the bulletin entitled "Mental Health in Later Maturity," USPH Service Supplement 168.

Finally this work of Stieglitz led directly to the creation of the research group in Baltimore under the leadership of Nathan Shock. The choice of Shock by Dr. W. Henry Sebrell to follow in this position with substantial financial support from Public Health Service funds and the collaboration of the Baltimore City Hospitals has meant a great step forward in creating a research unit for work with older people. As a by-product, the whole field of gerontology has been stimulated by Shock's interest in assembling information in the field, in editing the Macy publications upon aging, and in creating the excellent *Classified Bibliography of Gerontology and Geriatrics* published in 1950 by the Stanford University Press.

Furthermore, Stieglitz's association with the Macy Foundation and the federal government undoubtedly stimulated his thinking and broadened his horizon. Therefore, he has become one of the leaders, both through his writing and lecturing, in the United States. He has edited three editions of the clinical work entitled *Geriatric Medicine* and published *The Second Forty Years* which has been widely read by the general public. Furthermore, Stieglitz has advanced the thinking in many areas, such as the Medical Service of the Veteran's Administration, through his lectures and contacts.

To a lesser extent one could probably trace the influence of educational efforts of Macy Foundation leaders, especially Drs. Frank Fremont-Smith and L. K. Frank, upon about a third of those who have attended the annual conferences or assisted in writing *Problems of Ageing*. Another third of those who have been involved in these activities were men too near the end of life to exercise much leadership or be influenced in their own research activities by the Macy Conferences. Another third was composed of younger men who have been too involved in other interests to ever give specific attention to biological problems of aging. Undoubtedly, however, some of the thinking of even this group was conditioned by their discussions at Macy meetings, and even their teaching in various universities may have included some of the fundamental ideas that were reviewed each year.

From these meetings no one could certainly escape without realizing that the goal of gerontology was the creation of better health in old age and not the extension of the life span. No one could escape without appreciating tha[illegible] ent from th[illegible] and inescap[illegible] of pathological and age changes as well as the great variability of men and animals in old age due to the inter-

lication of *Problems of Ageing* also created some awareness that the biological changes of aging might be studied with profit to alleviate some of the ill health and dependency of the old. Thus, a new outlook was created that the "old horses" might be kept in the work harness for more years and thus insure more happiness, independence, and productivity. This might be called the biological impact upon the social sciences. The activities of the Macy Foundation such as backing the publication of *Problems of Ageing* undoubtedly influenced the viewpoint of the social scientists to a limited extent, even though the thousands of welfare workers who handle the aged may never have heard of the Macy Foundation and its activities.

Much of the vision of L. K Frank during his active years with the Macy Foundation is still bearing fruit. Frank had an unusual ability to see whole problems and to correct weaknesses in research activities. Thus, he visited our nutrition laboratory and soon realized that we were giving far too little attention to pathology. He corrected this by creating cooperative rsearch between nutrition workers in the Agriculture College in Ithaca and the Cornell Medical School in New York City. This cooperation still continues.

From the earliest times there has only been a limited amount of cooperation between nutritionists and pathologists because most nutrition research is concentrated in agricultural colleges, and pathology is found only in medical or veterinary fields. In the field of gerontology such cooperation is very important because no team is probably better suited to attack the problems than workers in these two specialized fields Neither can be very strong alone Frank a social scientist, appreciated this more readily than his colleagues in biology in medicine. Today this is still not understood by most administrators in either colleges of agriculture or medicine

Although the federal government had given much attention to financial problems of the aged and created a vast organization for pensions and relief, little attention had been devoted to medical problems until the Macy Foundation applied its unique stimulus about 1940. This stimulus represented a contribution in paying the salary of a specialist to serve within the U. S Public Health Service to create more interest in medical problems of the aged Fortunately, a physician of substantial vision and great vigor was selected for this position Possibly the vigor was too great for government agencies but it yielded some outstanding results during the brief period of activity of Dr E J Stieglitz

During his service in this position many achievements could be recorded, but three have resulted in permanent advances In the first place he summarized the activities of all researchers in the nation working in biological fields concerning aging. In the second place he

animals are in progress. The pressure is growing continuously to carry on long time studies of the effects of chemicals upon the animal and human body.

The constant stress of the Macy Foundation upon the importance of bringing many disciplines to bear upon large problems is also yielding results. The whole specialized field of nutrition has come to appreciate the merits of pathology. Interest in gerontology and the philosophy about the best ways of attacking its problems has undoubtedly spread to many other fields of science. In America especially there is an unusual interest today in cooperative research ventures in which scientists of very diverse backgrounds attempt to work upon a common problem.

It is true that the great problems of biological gerontology remain unsolved. No one has yet created facilities for providing supplies of old animals for biological research. No institute has yet come into existence where young scientists can devote their whole lives to research upon problems of gerontology with some degree of security Few long time research grants are available to scientists who desire to devote most of their efforts to gerontology Few young biologists are entering the field of gerontology because they do not wish to face starvation. Endowed institutions with teachers who can devote their leisure to research upon basic science are slowly being displaced by the same institutions manned by workers with vast research grants made by boards who look primarily at objectives of immediate utility Medical school administrators still have their eyes glued upon research projects dealing with diseased conditions and have little interest in projects in basic biology that attempt to determine the underlying causes of disease Most of the American public still accept the diseased conditions of the aged as an act of God with little chance of alleviation by any approach through basic science

However, the seeds of advanced thinking have been planted and the leading planter thus far has been the Macy Foundation, even though it has devoted only a modest fraction of its total resources to the field of gerontology

play between the normal and diseased body. No one could escape without realizing the hazardous nature of gerontological research as well as the immense patience and funds required if scientists were to labor in the field. Thus, teaching, lecturing, and thinking must have been modified in the case of everyone involved in the Macy Conferences.

If one looks further into the creative activities of the Macy Foundation in the field of gerontology, he comes to the birth of the *Journal* and Society in 1946. If one reads the certificate of incorporation of the Gerontological Society, he will note that every man had participated in the Macy Conferences Hence, one reads the names of Wm. deB MacNider, E. J. Stieglitz, R. A. Moore, Oscar Riddle, H. S. Simms, E. T. Engle, L. K. Frank, and Jean Oliver. In other words those who have carried much of the responsibility of getting both the organization and the *Journal* underway even to the present time are men educated in the annual meetings of the Macy Foundation. Furthermore, grants from the Foundation have kept the infant *Journal* alive until it could become strong enough to walk alone.

The Macy Foundation has had representation upon committees dealing with biological research upon aging within the federal government Thus we find L. K. Frank upon the committee of Thomas Parran, USPH Service in 1940. Frank Fremont-Smith as well as a number of others who had attended the annual meetings in gerontology of the Foundation served during the lifetime of the Gerontology Study Section of the Division of Research Grants of the USPHS National Institutes of Health.

Indirectly much of the thinking that crystallized during the fifteen annual meetings of the Macy Foundation dealing with gerontology has filtered into many foreign lands Italy now has two journals devoted to gerontology. In a recent number of one of these, *Longevità* January, 1953, one finds a rather complete review of the annual meetings of the Gerontological Society in Washington Thus thought flows out in many directions through many channels.

The future development of biological research in the area of gerontology might seem very dubious if one looked only at the immediate research areas in biology which are tagged with the label of gerontology. But if one looks at the over-all philosophy that has been created as a result of the fusion of activities of the Macy Foundation, the increasing public appreciation of problems of aging, and the growing concern of government agencies over the care of the aged, one realizes that genuine gains have been made in the field of biology. Today there is far more interest than there was twenty-five years ago in long time biological research. Long time studies of effects of radiation upon

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FRANK FREMONT-SMITH, *Medical Director*

JANET FREED LYNCH, *Assistant for the Conference Program*

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†Absent



MEMBERS AND GUESTS OF 1953 CONFERENCE ON PROBLEMS OF AGING

Front row Albert I Lansing, George B Wislocki, Roy G Hoskins, Anton J Carlson, F. Bourliere, E V. Cowdry, Warren Andrew *Second row* Bacon F Chow, Peggy Kubie, Janet Freed Lynch, Ephraim Shorr, Nathan W. Shock, Harold E. Himwich, Lawrence K Frank, Earl T Engle, Eyleen A Masser, Frank Fremont-Smith *Third row.* Henry S. Simms, George O. Gey, Robert J Havighurst, Jerome Gross, Frederick L. Hisaw

THE JOSIAH MACY, JR. FOUNDATION CONFERENCE PROGRAM

When I was on a destroyer out at Bikini in 1946 I was fascinated listening to our radio operator as he tested communication equipment. He would ask another ship through his radio, "How do you hear me?" and the answer often would come back, "I hear you Nine-Nine-Nine" That meant that everything was satisfactory. Of the three nines, one was for intensity, one for clarity, and one for meaning

The Josiah Macy, Jr. Foundation has organized and devoted a large portion of its resources to the support of its Conference Program because the officers are cognizant of the fact that there is considerable obstruction to communication and mutual understanding across the disciplines and specialties, and that this, in fact, is one of the major factors delaying scientific advance We feel that there are psychological, as well as semantic factors contributing to the difficulty of communication, people, even in arguments with one another, are too much inclined to make statements *at*, rather than to communicate *with*, others I think that we are inclined to forget, though, that the real question is, are these words and statements those which are likely to convey to the listener the whole or even a small part of what I would like to express

I have a feeling that we should be very much concerned with the other fellow's receiving set and not only with our own transmitter If the other person doesn't seem to understand us, it may not be enough merely to increase the power of our transmission; we must try to find the obstruction in his receiving set, and see what kind of filters and resistors he uses. So, if we call out to the interprofessional No-Man's-Land, "How do you hear me?" and the reply comes back, "I hear you Nine-Nine-Nine," we have the beginning of communication What we try to do in these conferences conducted by the Foundation is to set the stage for meaningful communication.

With the accelerating rate at which new knowledge is accumulating and with the increasing recognition that nature is of one piece, it becomes evident that the continued isolation of the several branches of science from one another is a serious obstruct-

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...points including nuclear physics at one end of the spectrum and cultural anthropology at the other, for

advances in one field are frequently dependent upon knowledge derived from quite another discipline.

Although the fertility of the multidiscipline approach is thus recognized, universities, and scientific societies and journals which are usually restricted to one small area of a field in their coverage, have not yet made adequate provision for channels of interdisciplinary communication. We do not wish to compete with the formal scientific meetings or with the scientific journals which have established patterns and formats for the presentation of material. Our purpose at the meetings is to keep an informal atmosphere and to encourage the exchange of methods, research plans, concepts and difficulties, which cannot be done if there is formal speech making.

The Foundation has endeavored to meet the need for interdisciplinary communication by bringing together for a series of two-and-a-half day annual conferences a small group of investigators, representing insofar as possible all the branches of science related to a chosen problem. Participants in these informal conferences over a five-year period develop a feeling of friendship, trust and mutual respect which in turn promotes communication, cross-fertilization of ideas and cooperation The success of such an endeavor, however, is dependent upon full participation of all members in the discussion. Accordingly attendance at any conference is limited to twenty-five.

Under the guidance of Dr Willard C. Rappleye, President of the Foundation since 1942, the Conference Program has been gradually expanded and enlarged until during 1953 it included twelve different groups which meet annually to discuss a wide variety of problems in the field of medicine and the closely related disciplines Our plan is to discontinue the meetings of each group at the end of five years.

In order to share with a wider group of investigators and students the essential quality of these conferences and to give others an insight into the functions of the scientific mind, the informal nature and tempo of the discussions, as far as possible, are preserved in the published transactions.

FRANK FREMONT-SMITH, M.D
Medical Director

INTRODUCTORY REMARKS

ROY G. HOSKINS
Chairman

THE WORK of this conference of the Macy Foundation has, I suppose, as its ultimate motivation, the improvement of the state of the elderly human being The problem of aging, of course, presents a number of facets, more or less distinct, often greatly overlapping These facets have been approached in a variety of ways, sometimes in a rather hit-or miss fashion, sometimes more systematically. I like to think of man as an integrated organism that is susceptible to study at a considerable number of emergent levels He can be thought of as a psychological organism

Then, one's interest is focused on the intrapersonal characteristics, especially the ancestral drives, the indulgences and frustrations. A great deal of human welfare hinges on those issues

He can be thought of at the social level, in his relationships to the environment, and especially the human environment His interpersonal relationships come into that

We have dealt with man at the social level, considering his relationship to other people and to society That involves a good many economic issues of one sort or another We have dealt with those somewhat systematically, at a previous conference. We have also covered problems of nutrition and consumption.

There is one aspect that is rather notable for its omission, and that is man and his cosmic environment, his cosmologies and theologies. By common consent, we have stayed off that ground It has never been quite clear in my own mind why we have, but we have

We have dealt with the organ systems which, at the physical level, make up the individual The kidneys and skin are two of the organs that have had somewhat special attention, depending, again, I think, especially on the personality of this group In the years during which Dr MacNider was the moving spirit, we heard a great deal about the kidney, because that was the field in which he had worked Dr Cowdry has often directed attention to the skin

At the present conference, we have logically come down pretty well to the foundation level that is to say, the anatomy and physiology of the cell I think it might be desirable if we stick fairly closely to the cellular level, both as to structure and function. Perhaps it is wise

that we do come to this level of consideration at this time, because in the past decade, as, of course, you all know, there has been very much advancement in instrumentation. We have electronmicroscopy, which is developing, the finer cytologies, tissue cultures, and things of that sort. We know a great deal more about enzyme chemistry. Then there have been important developments in comparative biology, you might say, things we may learn from other organisms, rather than man himself. The genetic aspects, again, come into the picture.

In proceeding from this point to the development of the thought we may have, I think we would be wise to stay pretty well by the old pattern that has gone through these discussions throughout all fifteen years, the aim being not so much to determine what is known, as what is not known, and how we can most wisely proceed to fill the gaps in our knowledge.

That would logically bring up some discussion of the newer techniques and what these techniques can teach us The potentialities and weaknesses of these techniques would be other problems that would logically come in at this time.

Before proceeding with our discussion it may be well to become briefly autobiographic, and introduce ourselves Nathan, will you start the ball rolling?

NATHAN W. SHOCK: I am Chief of the Section on Gerontology of the National Heart Institute, National Institutes of Health Our research laboratories are operated in collaboration with the Baltimore City Hospitals in Baltimore After receiving my M.S. in organic chemistry at Purdue University, I completed my graduate training at the University of Chicago under Dr A J. Carlson, Dr A B. Hastings and Dr. L L Thurstone. My major research interest was the evaluation of physiological and biochemical factors influencing behavior After completing my graduate work, I spent ten years at the University of California with Professor Harold E. Jones working on the physiological aspects of adolescence and teaching physiology in the University of California Medical School. In 1941 I accepted an appointment in the Unit on Gerontology which had just been established in the National Institutes of Health by Dr. W. Henry Sebrell I came to the position with no real knowledge of aging, but thanks to Dr Cowdry's excellent book and the conferences on aging, sponsored by the Macy Foundation as well as the continued support of the Public Health Service, I found the field most stimulating and of widening interest.

EPHRAIM SHORR: It was just about three months ago at another Macy Conference that I had to provide reasons for having become interested in circulatory homeostasis; and now if I provide equally good and sufficient reasons for my interest in gerontology, I hope it will not appear that I am fickle in my attachments.

Specifically, my interest in this field may be said to stem from my relationship to Frank Fremont-Smith and the Josiah Macy, Jr. Foundation. About twenty years ago, when I was asked to set up an Endocrine Clinic at The New York Hospital, I began with Dr. Papanicolaou to use vaginal smears as objective indices in the study of disturbances in menstrual function in women. A few years later, discussions with Dr. Fremont-Smith of the problems involved in the menopause, stimulated a more extended exploration of the various metabolic changes which occur in this period in the life cycle of women. This led me to an interest in osteoporosis, and in the aging of the blood vessels in relation to hypertension. At about that time, I was invited to join this group. The stimulation that has come from these meetings, the investigational assignments which stemmed from them and the associations formed here have all been factors entering into my orientation towards gerontology.

E. VINCENT COWDRY: I came into the study of aging by way of arteriosclerosis. About 1932 Dr. Ludwig Kast came to Washington and asked the Division of Medical Science of the National Research Council about the advisability of preparing a comprehensive statement concerning arteriosclerosis. He was a pioneer, I think, in realizing that the time for the concentration of attention on chronic disease had arrived, that we must work on a long-term basis, and that the preventive medicine of chronic disease should be our goal. The acute diseases had been tackled pretty successfully, while the chronic diseases had been neglected. That was his first point of view.

His second point was that this statement must not be narrow, but that it must represent all aspects of the problem that could properly be presented, and that it must be international. Thus, we benefited by the cooperation of scientists in England, France, Germany and Russia. It was the first international study, as far as I know, of arteriosclerosis.

I should like to take a few moments to trace the sequence of developments, if I may. This work on arteriosclerosis showed that it was desirable to broaden the basis of our in-

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that we do come to this level of consideration at this time, because in the past decade, as, of course, you all know, there has been very much advancement in instrumentation. We have electronmicroscopy, which is developing, the finer cytologies, tissue cultures, and things of that sort. We know a great deal more about enzyme chemistry. Then there have been important developments in comparative biology, you might say, things we may learn from other organisms, rather than man himself. The genetic aspects, again, come into the picture.

In proceeding from this point to the development of the thought we may have, I think we would be wise to stay pretty well by the old pattern that has gone through these discussions throughout all fifteen years, the aim being not so much to determine what is known, as what is not known, and how we can most wisely proceed to fill the gaps in our knowledge.

That would logically bring up some discussion of the newer techniques and what these techniques can teach us. The potentialities and weaknesses of these techniques would be other problems that would logically come in at this time.

Before proceeding with our discussion it may be well to become briefly autobiographic, and introduce ourselves Nathan, will you start the ball rolling?

NATHAN W. SHOCK: I am Chief of the Section on Gerontology of the National Heart Institute, National Institutes of Health Our research laboratories are operated in collaboration with the Baltimore City Hospitals in Baltimore After receiving my M.S in organic chemistry at Purdue University, I completed my graduate training at the University of Chicago under Dr. A. J. Carlson, Dr. A B Hastings and Dr L L Thurstone. My major research interest was the evaluation of physiological and biochemical factors influencing behavior. After completing my graduate work, I spent ten years at the University of California with Professor Harold E Jones working on the physiological aspects of adolescence and teaching physiology in the University of California Medical School. In 1941 I accepted an appointment in the Unit on Gerontology which had just been established in the National Institutes of Health by Dr W Henry Sebrell I came to the position with no real knowledge of aging, but thanks to Dr Cowdry's excellent book and the conferences on aging, sponsored by the Macy Foundation as well as the continued support of the Public Health Service, I found the field most stimulating and of widening interest.

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His second point was that this statement must not be narrow, but that it must represent all aspects of the problem that could properly be presented, and that it must be international Thus, we benefited by the cooperation of scientists in England, France, Germany and Russia It was the first international study, as far as I know, of arteriosclerosis

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For a number of years I have been interested in the biochemistry of growth. Well, we know an animal grows when he is put on a good diet. But what do we mean biochemically when we say animals grow? They increase in body weight, they increase in length, and they increase in a number of other things. We tried to go into a little more detail by studying the changes in the body composition and, in particular, some of the enzymes and proteins Later on we studied the biochemical changes due to stress. When we looked the data over, there seemed to be a common denominator as far as the biochemistry of growth and stress was concerned. And in thinking it over, growth is one phase of life, whereas aging is another phase of life.

Isn't it, therefore, conceivable that some of the knowledge we have gained about the enzymes and the composition of the body could be applied to the process of aging?

ROBERT J. HAVIGHURST: After completing graduate work in the fields of chemistry and physics, I became interested in education, and taught in experimental educational programs at the college and secondary school levels for several years Then I joined the staff of the General Education Board (Rockefeller Foundation) and soon became interested in child and adolescent development, largely under the stimulation and guidance of Lawrence Frank When, in 1941, I went to the University of Chicago, I knew then that I wanted to be interested in human behavior and human development throughout the life cycle At Chicago my research has been in the area of social psychology of children, adolescents and adults As a member of the Committee on Human Development at the University of Chicago, I work on an interdisciplinary team with people from the departments of psychology, anthropology, sociology, physiology, and education When I learned

that the Social Science Research Council had set up a committee on social adjustment in 1945, I talked with Professor Burgess, who was chairman of that committee, and we agreed that we would try to establish a subcommittee on social adjustment in old age He and I together developed a program

I think I am the only social scientist in the group here, and for the purpose of this conference, I believe I ought to remember that I took my degree in chemistry I shall try to be a chemist for the next couple of days.

ALBERT I LANSING: I am a member of the staff of the Washington University School of Medicine I received my training in zoology, have done most of my work in cell physiology, and make my living as an anatomist. But throughout these three fields, my activities in problems of aging have carried through as a thread As a matter of fact, my interest in gerontology goes back to quite a long time ago. As a junior in high school, I was very much impressed
the concept of intestinal pu
on I did experiments on m
ilus as a means of altering
whether they will delay my demise or not

But, speaking of gerontology, I think I should point out that I have a very soft spot in my heart for both Dr Frank Fremont-Smith and the Macy Foundation I don't know whether all of you are aware of the fact that the interest of Dr Fremont-Smith and the support of the Macy Foundation made possible in 1941 the beginning of my career as an active gerontologist with Dr Cowdry It was directly implemented through that support

FREDERICK L. HISAW I think probably for this discussion my remarks ought to be limited to things related to the aging process

By training and thought, I am a zoolog ... I am particu-
When I was
group a num-
... years ago, I had several rather long talks with Dr. MacNider concerning what contributions if any I might be able to make to the group

A number of things were mentioned at that time One of these, of course, was the fact that the physiology of reproduction has a very definite relationship to aging: coming into

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I was working in endocrinology at the time I was invited into this group, and also was engaged in research on schizophrenia at the Worcester State Hospital It was perhaps because of my endocrine interest that I was invited to come aboard However, being with the group and watching its development has been a very delightful experience Even at that time the subject

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sexual maturity, functional reproductive life, and then weakening of reproductive processes, had a direct bearing on questions of aging. And then, more particularly, the point I presented, which he thought was most worth while, was my interest in the aging of certain tissues. There are certain tissues, such as the corpus luteum of the ovary, the placenta, and other tissues associated with the reproductive process, which come into existence at each estrous cycle or pregnancy, and which have a very definite functional history, including changes associated with age and finally senility and involution and disappearance.

So, I have been quite active for the last fifteen years in an attempt to study what it is that brings a tissue into its functional existence, what it is that maintains the physiological process, and why it is that the physiological action of a particular structure finally ceases. To me it seems quite probable that, if one could get an idea as to the nature of the aging process in a particular tissue, it might contribute to a better understanding of the aging process in general.

So, if I have made a contribution to this group, it has been along those lines.

JEROME GROSS: I am associate biologist in Dr. Walter Bauer's Arthritis Study Group at the Massachusetts General Hospital and am also research associate on the staffs of the Department of Biology, MIT, and the Department of Medicine at Harvard Medical School

My interest, primarily, is in the molecular and colloidal organization of tissues and their reactivity, with particular reference to connective tissue I became interested in this field in medical school, maintaining an interest in rheumatic disease and in the application of physical and chemical principles and methodology to biological problems My interest has not been primarily gerontological However, our studies on the development and alteration of connective tissue and fibrogenesis, and of collagen have some bearing on the widespread mesenchymal changes in aging processes and degenerative diseases

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When I went to Brown University to do graduate work, good many people were sitting about at microtomes havir a great deal of trouble sectioning material and putting it c slides, and I decided definitely that I would not go into hi tological or cytological work, so I began to work on th problem of the senescence of Cladocera, and studied the wan ing of their reproductive capacity as evidenced by the increas ingly smaller number of babies that could be found in the jar of these animals from one day to the next. I began to notic some morphological changes with age But the very next year I had started in histological work, because it was an intriguing thing I had to do my own technical work, of course, and was using the microtome. This gave rise to a Ph D. problem in zoology.

After I had finished my graduate work and had begun teachit [illegible] w [illegible] *Problems of Aging* had been published. This gave me an impetus because at that time I had somewhat of a feeling that studies on aging were not entirely "respectable" in scientific work When I saw that such a fine group had banded together to publish this book and that many people were definitely interested, and in a fundamentally scientific way, I felt it was quite in place to go ahead.

Then, in 1941, the Wistar Institute of Anatomy started on a project. It was rather interesting in showing the need for studying aging because Dr. Farris wanted to study the effects of emotional crisis in the lives of rats, a crisis brought about by a terrific noise directed at the rats He wanted to see what would happen to the animals throughout their life histories as a result of this type of crisis. But he found that he had no control material because nobody knew exactly what happened to the tissues of rats as the rats grew older normally, so he asked about twenty to twenty-five people to collaborate in studying the changes in the normal tissues with age That greatly appealed to me, and proved to be a very helpful thing for my own work With the possibility of having graduate students, which came up about four years ago, I felt that it was possible to expand a little and get into some of the cytological problems that had been appealing.

I occasionally dip back into less formal literature on aging, and must admit that about a year ago I read a rather fanciful book called *Old Age: Its Cause and Prevention*, which was written by a San Francisco author in the early part of this century, the main gist of which was that he had discovered a system of exercises in bed which were guaranteed to prevent or defer for a very long time the onset of aging. He had a before-and-after picture of himself He looked like a rather senile and run-down character at 51 years of age, and like a very spry and agile person at 75.

So the fundamentally scientific aspects of aging have been fascinating to me. One aspect of studies of aging that has appealed to me all along is that the field and the various ramifications of it have no apparent end,

BOURLIERE· I am associate professor of medical biology in the Faculty of Medicine of the University of Paris. A naturalist by avocation, I got my M D degree in the University of Paris in 1940 and afterwards graduated in physiology, biochemistry and animal biology in the Faculty of Science of the same university. During my internship in the Necker Hospital in Paris, I became interested in gerontology when working with Professor Binet, the present dean of our medical school, whose interest in old age problems is very great He encouraged me to devote most of my time to gerontological research, and the establishment of a new department of medical biology in our medical school gave me the opportunity to start research of my own on the comparative physiology of growth and aging

My present work is mainly concerned with the comparative study of growth and aging patterns in cold-blooded vertebrates, as compared with warm-blooded vertebrates, and with the study of those mammals whose poor temperature regulation is associated with a very long life-span Our approach is both ecological and physiological and I hope it will some day become biochemical

ANTON J. CARLSON: I have worked in physiology as a member of the faculty of the University of Chicago for 48 years. Of course I realized very early, long before most of you were born, that the basic problems in biology and in disease all bear on the unsolved problems of aging — all of them, including basic

problems in sociology. Back in 1912 or 1913 I carried out a series of experiments on the human stomach. Extending that work, I found in checking the hunger contractions of the empty stomach of adults against the hunger contractions of premature human infants, that the period of rest of the empty stomach in the infant was only about five or ten minutes, and that in most of the adults it was from two to three hours, and *the older the individual, the longer the rest period between* the hunger contractions. That was not only true of man, but also of the dog. That is a phenomenon of functional aging, and so is the decrease in gastric secretion

One of the last pieces of work of interest to me was to challenge some experiments by one of our members, Dr. McCay. He had controlled rats, litter mates, and he fed some of them so little that they grew very slowly, but their life span was much longer than that of their brothers and sisters who had received as much food as they wanted. I visited my friend, Dr. McCay, at Cornell University and asked him, "Do you know whether you have really lengthened the life of these undernourished rats or whether the life of the rats who *just ate and sat, without any struggle for food*, was shortened?" So we did experiments on periodic fasting, or putting so much indigestible roughage into the food that the rats couldn't overeat, and that lengthened their life

I have profited by association with this group. This time, however, I came here, Dr. Fremont-Smith, essentially as a guinea pig, because I think I am the oldest in the group. One of you—I think it was Cowdry—says that old people are bored If we could maintain our primitive curiosity, we would not be bored We would enjoy learning new and important things as long as our cerebrum works And that is one of the big problems, I think, in adding years to life in a group like this, in the Gerontological Society, and in the International Congresses of Gerontology

GEORGE O. GEY: I am at The Johns Hopkins University. I began my scientific career over thirty years ago at the invitation of two people, first with Dr Clarence C Little, of Harvard University and director of a program of experimental cancer research at Cold Spring Harbor, where I was one of his assistants in 1921, then with Dr Edward Adolph, who had just come from Oxford. At that time, I was a graduate student at the University of Pittsburgh, where Dr. Adolph was teaching zoology and physiology, and I was one of the instructors. I

was also one of his early water-balance guinea pigs. Following my early contacts with these men, I landed at The Johns Hopkins Medical School and was invited to work with Dr. Joseph C. Bloodgood on tumor pathology and on the cultivation of tumors (1) in the laboratory of Dr. Warren Lewis, of the Department of Embryology of the Carnegie Institution of Washington in Baltimore To my knowledge, this was his first sojourn in tumor cytology Dr Lewis was one of the leading cytologists of the day. I have received much stimulation from him since that time.

One of the problems at that time was this business of isolating in culture cell strains so that one could study these target end organs, so to speak, outside the body. For years using such biological material (i e, cultured cell strains), Mrs Gey and I have been faced with the problem of serving the interests of many For a while it seemed as though one of my chief functions in life was to direct a laboratory with cultured cell strains to help satisfy the scientific curiosity of others besides myself (2).

After a year at Hopkins, working with Dr Lewis, I moved to the Columbia Hospital in Milwaukee, Wisconsin, with the encouragement of a host of Hopkins people who were there; especially Dr George L Streeter, Dr. Joseph C. Bloodgood, and Dr Warren H Lewis These men encouraged me to work with one of the Hopkins men, Dr John L Yates, then in Wisconsin I spent a sojourn of some six years in Wisconsin where I set up a cancer research laboratory at the Columbia Hospital in Milwaukee Through correspondence and direct contact, I got much guidance from Dr Ludwig Hektoen, Dr Preston Kyes, Dr R R Bensley, and Dr. A Maximow This encouragement from the group at Hopkins and of local men in Wisconsin, especially Dr Bunting and Dr. Thalhimer, and contacts with Dr Alexis Carrel and Dr James Murphy of the Rockefeller Institute, enabled me to continue, even in a period when the kind of things that we were doing were quite unpopular, at least in some quarters

Since that time, I have continued to use primary explants of tissues and isolated cell strains maintained in continuous cultures as our chief objects of study These cells can readily be chosen from young and old hosts, and it is, of course, a natural thing to ask whether these isolated cells are fundamentally different from one another. Because my support came chiefly from organizations interested in cancer biology,

I quite naturally kept my main ideas focused in that direction.

However, my connection with gerontology, in the immediate past, came from an invitation from Dr. Korenchevsky, of Oxford, and from Dr. Cowdry to participate in the Second International Gerontological Congress held in St. Louis in 1951. I must say I got a lot out of that meeting. I was faced at that congress, as most of you were, with more problem identification, and I am pleased to say, this no longer constitutes a big issue in my mind. At an earlier period we had too little of it in our laboratory. Improved methodology plus a long experience has made problem identification easier. As a result, we have found our kind of activities more popular.

We have tried to identify specific facets of interest so that we can work along definite lines, often backing individuals to pursue them rather than less well-defined projects. This seems to me the most economical way to do it, to get a good man who has a well-defined problem, and let him run it down to a satisfactory conclusion. Often-times lack of continuing support interrupts such research.

We have had many young scientists from all parts of the world in our laboratory. For example, one of this group has worked directly on problems related to aging. He is Dr. André Glinos, who has been studying the problem of the relationship of regeneration and neoplasia and has done some good work on the subject. I have always been interested in the relationship of aging to cancer and, therefore, sponsored this work with much interest. My personal belief is that there is no direct relationship of cancer to aging.

Malignant transformation can occur at a very early age in development. From the blastocyst stage tumors can arise from the extra-embryonic trophoblast resulting in the production of chorionepithelioma. From here on tumors arise from the tissues at all ages, but induction of them may require many years as compared to the briefer periods at the very beginning of life.

At the present time, Dr Bang and I are much fascinated with our work on viruses. As you know, viruses are organisms which require cells for their existence, and I believe that viruses have a lot to do with some of the processes of aging. Now, there is another facet of our work that I am greatly interested in, and this is the thing we call dormancy in tissues and in the organism as a whole. Dr Glinos is much interested in this, also.

There are many situations in which, after regenerative repair, the whole tissue responds to an influence which we can poorly define. This influence, which we see in the host, is manifested in the form of maintenance of differentiation and a settling down It is something which one can manipulate in a test tube of cultured tissues. To get at this, one must, of course, fractionate the humors of the organism in order to understand what goes on in the host One cannot do much of this sort of thing very easily in the host, but one can do it easily, we feel, in the test tube

We have been very fortunate in having some altered cell situations arise in our culture work, and I should like to say briefly what they are and why we should pursue some of these lines of approach We have seen the production of malignant cells in what were previously permanent and stable lines of normal cells We are not able to account for these transformations into malignancy Nor are we able to say that this is the only way in which a cell can develop when it deviates from its normal state Someone here spoke of the flattened cells that occur in the kidney and the liver and how different they are from what they were before. Well, I *can* assure you that after a radiation exposure, cells *are different* from what they were before, and it is understandable why that should be the case The mutagenic effects of injurious agents are well recognized

In other work that we are interested in, we have seen the loss of transplantability of a tumor cell strain, or, one might say boldly, the loss of malignancy, in cells that once were malignant This is a process that we do not understand at all

If one were to think of our laboratory as a bacteriological laboratory, one might readily understand some of these things, if we substitute micro-organisms for cells, because in the field of microbiology, all kinds of transformations are found. To some extent, by manipulation, transformations can be produced, some rather transitory, others ending in permanent mutations

In all of our work, it has been necessary to resort to improved methodology, and we have tried to contribute in this direction. More recently, we have developed a new method for growing cells in suspension, so that those who are interested in direct chemical analysis or other studies of large masses of cells, can carry out some of these procedures

HAROLD E. HIMWICH: I am a physician and a physiologist and so, like

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HAROLD E. HIMWICH: I am a physician and a physiologist and so, like

most of the people here, I am interested in the field of experimental medicine. My methods have been chiefly biochemical. I have been working for some years in the field of brain metabolism and about a year ago I was asked to take charge of the research program of the Galesburg State Research Hospital at Galesburg, Illinois. Because of my interest in neurophysiology I have also been made a member of the Department of Physiology of the University of Illinois School of Medicine.

Almost all our patients are in the latter part of the life span, and a high priority in our research project is concerned with old age and mental disease of old age. My new responsibilities make me appreciate all the more the opportunity to attend this meeting.

At the present time we are feeding very large doses of glutamic acid, higher than previously given in most instances, to 32 patients and to a group of normal controls who are members of the research staff. I won't be able to tell you anything about the results now, because only the dietitian knows how and when glutamic acid is being given to the various subjects. In fact, we shall not begin the analysis of the results until early next summer. But I should like to say one word or two on the rationale of this experiment.

Although endogenous glutamic acid is oxidized by the brain, there is fairly good evidence that it doesn't enter that organ rapidly. It penetrates the blood-brain barrier too slowly to support the rapid rate of brain metabolism

About three years ago, Weil-Malherbe (3) suggested that glutamic acid is adrenergic and induces a prolonged, physiological stimulation of the adrenal medulla. There is some evidence in favor of that possibility during hypoglycemia since his results were obtained on patients receiving insulin therapy for schizophrenia He reported an increase of adrenaline-like substances in the blood when glutamic acid was given during this treatment Blood sugar rose, blood pressure increased and pulse rate was accelerated. That is the basis on which we started our work

I should like to say that we are avoiding the controversial subject as to whether or not glutamic acid affects the I Q. We are also omitting all projective tests Ours is a multi-discipline attack in that we are using psychophysiological, physiological and biochemical rather than other types of tests. Evaluations of the effects of our treatment upon personality structure, however, are being made by a psychiatrist.

HENRY S SIMMS· I am at the College of Physicians and Surgeons of Columbia University, New York City. My training was in biochemistry but I am located in the Department of Pathology.

My interest in gerontology began 25 years ago here in the town of Princeton. At that time I was working at the branch of the Rockefeller Institute that was located on the outskirts of Princeton, and I was doing physiochemical work on proteins and amino acids, but I felt that I would much rather change my field of research and work on more important problems. It seemed to me that th [illegible] l [illegible] s [illegible] problems. I have been doing so for the last 25 years, but haven't solved either of them yet

I have been thinking, during the conversation here today, about the difference in the situation 25 years ago relative to the problem of aging as compared with the attitude toward gerontology at the present time. Twenty-five years ago, as Dr Andrew pointed out, working on aging was not considered respectable. There was practically no scientific interest in aging It was practically impossible to get financial support, because the foundations at that time were handicapped by shortage of funds and were forced to spread their money out for research on problems that could be worked out in a year or two There was less interest in long-term projects.

As far as the public was concerned, the attitude toward research on aging was, "Well, why not let the old folks die? Why do research to keep the old fossils alive?"

As for the work which had been done previous to that time, much of the work was in support of crackpot ideas, but a good deal of it revolved around the idea that if we knew enough about regeneration of organs and tissues, we could solve the problem of aging. That was a nice thesis, but it hadn't solved the problem of aging A great deal of work had been done on regeneration in lower animals with the hope that it might throw light on the problem of aging.

But, during the past 25 years, there have been changes in the attitude of the public, there have been many changes in the attitude of those who support research, and we who are interested in the field of aging have, I think, gotten a much better conception of the aging problem even though we have not solved it yet

I feel that the Macy Foundation has done a great deal to

promote this field. The Gerontological Society is an outgrowth of the Club for Research on Aging, which received support from the Macy Foundation. And the Gerontological Society, as we all know, has developed into a large organization. I feel encouraged that, although aging is a complex problem and a long-term problem, we are making progress in its solution.

GEORGE B. WISLOCKI: I was brought into this group at the invitation of Dr. Aub about ten or twelve years ago. I must admit that up to that hour I had not taken any very active interest in gerontology or considered that I was going to. I would not put down gerontology or aging as one of the topics that has received much attention in the way of research in my laboratory.

There are two things perhaps which give me a slight claim to knowing a little about some manifestations of aging. These are, one, an interest together with Dr. Aub in the growth of deer antlers and the endocrine factors which control them, and second, my interest in the growth and aging of the placentas of humans and various animals

Deer antlers and placenta are organs which are favorable for the study of aging because they encompass their growth, maturation and involution in a relatively short time, besides which they offer an opportunity for analysis of their growth and age changes in terms of endocrine factors which regulate them.

Aside from these special phases of aging, I have given no real thought to the problem. However, I have learned a great deal about aging from listening to the experts in this conference group I have become familiar, on the one hand, with the very important practical issues of aging in the human race, including the care and guidance of the aged, their economic and social status and the illnesses to which they are subject. And, on the other hand, there are far more difficult and elusive problems of the biology of aging, involving the chemical and physiological factors which control the destiny of tissues At these levels relatively little has so far been elucidated about growth and aging or about the related nature of cancer.

I have made several interesting contacts with members of this group. I have arranged for Dr. McCay and Dr Charles P. Lyman of my laboratory to study how hibernation affects aging. They are attempting to find out whether, if hamsters

are kept maximally in hibernation throughout their lives, their lives will be lengthened.

Two of my associates who have been studying hibernation in hamsters have initiated some work on cancer. Because it has been shown that cancers can be transferred and grown in the cheek pouches of hamsters, Dr Charles P. Lyman and Don W. Fawcett became interested in what hibernation would do to such transplants. They have demonstrated that while the animals are in hibernation, the growth of the cancer transplants is inhibited However, the transplants are not completely destroyed, for as soon as hibernation is terminated, they begin to grow

Perhaps the most valuable stimulus that I have obtained from contact with this group has been to become acquainted with the atmosphere of these interesting conferences The free interchange of ideas at a very informal and relaxed level makes possible the crossing of lines between disciplines, to a degree which has not been achieved by other types of groups who have maintained their relationships at a more formal level That has so impressed some of those who have participated in Macy conferences that the pattern established here has been initiated elsewhere

EARL T. ENGLE: I think historically, that as others here, I became interested in this problem when Dr Cowdry was organizing the first edition of his very well-known book on aging. In the process of development of that, I became a contributor. Then, of course, that dynamo, Korenchevsky, came over on a whirlwind tour, and after conferences over the country, and guided by the great foresight and leadership of the Macy Foundation, the aging club was started. Not only has one been greatly stimulated over the years by the factual material that is presented here, but also there has grown up and continued that wonderful feeling of good fellowship, the sharing of opinion and point of view that has gone on I shall regret very much if this study group has no further sessions, but I shall certainly remember it with great pleasure all my life

CLIVE M MCCAY. I am professor of nutrition at Cornell University. My interest in aging goes back a quarter of a century, when I was a research fellow at Yale. In one of Professor L. B Mendel's seminars, we discussed the problem of retarded growth in rats I asked Professor Mendel if retarded rats would have longer spans of life He replied, "You are young,

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CELLULAR STRUCTURE

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Department of Anatomy
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Wake Forest College

I SHOULD LIKE to begin by expressing my very warm and sincere thanks to the officers of the Josiah Macy, Jr. Foundation, and to the organizers of this program, who made it possible for me to be here. I hope that I shall be able to follow the pattern, which is most desirable for this sort of conference, and, while bringing out some facts, aim particularly at the raising of questions and pointing toward the things that we do not know.

I believe it is still admitted that the cell can be considered as a common denominator of living things This is in spite of the fact that there are changes in zoological theory, some contentions that Protozoa should not be considered as single-celled animals and that there are increasing numbers of examples of animals in which not all the organism is cellular, but in which a great deal of it is syncytial It is still admitted, too, in spite of the fact that we have pushed a long way into the ultramicroscopic field and have gotten so far below the cellular level that sometimes we do not recognize that the things we are looking at are actually within cells

The cells are important to us in the study of aging, it has seemed to me, because they are a common denominator, not particularly because they are small, since the fact that something is smaller than something else does not mean necessarily that it is more fundamental

If we had the problem of studying changes in cells with age, in an organism, such as, say, the sponge, in which there are only a few simple types of cells, which after dissociation can actually come together and within a short time form a whole new organism again, perhaps our studies would be less involved But, of course, we are primarily interested in man and in the other higher animals, and here we have immense aggregations of cells Somebody has estimated that there are 26 trillion cells in the human body, and I, for one, am willing to take that figure, since I have not seen a better one That is 26 million times a million cells

These cells differ very much qualitatively There are many different classes and groups of cells, which differ not only in their form and size

try it." I have spent a quarter of a century "trying it."

A second factor that intrigued me resulted from our studies of growth of trout, started in Connecticut while I was a student at Yale in 1925-27. We observed that slow growth in trout resulted in longer-lived fish. This led to a long series of studies with trout that ran from 1927 to 1943. We never learned the basic reason why slow growth in trout or any other species extends the span of life. From trout and rats, we gradually broadened our interests to include the whole field of gerontology The most important influence in increasing our interest was the stimulus from the meetings of the Macy Foundation and acquaintance with the men brought together for these meetings

LAWRENCE K. FRANK: My concern with the problems of aging began in 1923 when the Laura Spelman Rockefeller Memorial undertook to foster the study of child growth and development, focusing initially upon the pre-school child. This program, continued later by the Spelman Fund and General Education Board was enlarged to include the study of pregnancy and prenatal development, of infancy, of puberty and adolescence. Then in 1936 the Macy Foundation, on the initiative of Dr. Ludwig Kast, began to foster studies of aging and the aged, including support to the first systematic presentation on aging edited by Dr. E. Vincent Cowdry.

It has been my great privilege as a Foundation executive to participate in the planning and financing of these ramifying studies of human growth which I believe constitute one of the most significant and fruitful fields of contemporary research and service

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These cells differ very much qualitatively. There are many different classes and groups of cells, which differ not only in their form and size

and structure, but also very much in their function, and in their physiological attributes.

A number of the persons who were first interested in changes in cells in old age studied nerve cells. Back in 1894, Hodge (1) studied the nerve cells of newborn infants, middle-aged men, and old men. He also studied the changes in the ganglion cells of the honey bee, where he also found differences with age.

When we became interested in changes in old age, we thought it would be well to start out on some ground that was already reasonably firm, and since there were papers on this subject of changes in the nervous system, we began to study the literature from that field, to see what changes could be found.

Fremont-Smith: Dr. Andrew, do you have a rough estimate of the number of cells in the human nervous system?

Andrew: No, I don't. I have seen the figure of 12 billion for the cerebral cortex.

Hoskins: The over-all number which you gave also includes the blood elements, does it not?

Andrew: Yes, all the cells of the body, but I do not know whether in that calculation the muscle fibers were figured as so many cells, according to the number of nuclei in the syncytia

Gey: The number of cells for the whole organism has been carefully calculated by Dr. Warren Lewis. I have forgotten the number. I believe your first figure fits in with that.

Andrew: That may well have been the source

I began to study the cells of Purkinje in the cerebellum. This was a nice subject for study, because other workers, including Dolley (2), had studied these cells He had worked with them in the dog; Spiegel (3), in Germany, had worked with them in the guinea pig; Inukai (4) had studied them in the white rat; so I wanted to see whether their findings could be confirmed, and also, what new things could be found.

These cells, you remember, are so nicely arranged along the surface of the complicated "arbor vitae" of the cerebellum that we can study them in a fairly systematic way, and can make counts and compare one group of cells to another

Figure 1 shows the Purkinje cells of the cerebellum in young (*A*) and old (*B*) mice In a young mouse, we see a large amount of basophilic, or Nissl, material in the cytoplasm, and a quite clear background of the nuclear sap In senile mice, on the other hand, mice of 702 days or more, the cytoplasm generally contains considerably less of the basophilic material, and there is a tendency to a somewhat greater staining capacity on the part of the nuclear background (5)

In mice, also, we found the presence of amitotic divisions, dumbbell-



FIGURE 1 Comparison of the Purkinje cells of young and old mice (A) Cells from a young adult animal, 163-days of age (B) Cells of a senile animal, 702 days of age There is a general tendency to diminution in amount of Nissl material and an increased staining reaction of the nuclear sap in senility Reprinted, by permission, from Andrew W The Golgi apparatus in the nerve cells of the mouse from youth to senility *Am J Anat* 64, 351 *(1939)*.

shaped nuclei in some of the Purkinje cells, in about three or four per cent of them, and then the presence of binucleated cells, which were the result of this type of division. We did not find that until we had studied the Purkinje cells for some time, although we had read about Inukai's finding of binucleate cells in the rat. Therefore, we were very pleased to be able to confirm some of these things.

Then we went on to other parts of the nervous system.

Figure 2 shows the semilunar ganglion of a young (*A*) and old (*B*) mouse, and again we see this very heavy basophilic material in the cytoplasm in the young animal, and the extremely clear nuclear background, whereas in the senile animal, we have much less of the basophilic material, and we have a nucleus with the deeper stain. Also, in general, the cells of the old mice have lighter-staining nucleoli.

Simms: How old are the animals in the younger group?

Andrew: This particular animal was only 40 days of age. The Purkinje cells which were shown before as being from a young animal were from an animal of 163 days.

Wislocki: How was this particular preparation stained?

Andrew: This was stained with cresyl violet, one of the Nissl stains, without a counterstain

The changes in the nervous system seem to be frankly degenerative. The cells seem to be growing old themselves, we might say, and degenerating, and some of them are dying. Spiegel had made counts on the Purkinje cells in the guinea pig and Inukai in the rat, and they had found a decrease in the number of cells, which supposedly could mean only that they were degenerating and being dissolved.

Carlson: What kind of control did he have there as to number? He could not have a control in the same animal.

Andrew: Of course, he could not have litter mates, either. But he did have other guinea pigs of supposedly similar genetic constitution.

Carlson: So far as number of cells was concerned?

Andrew: Yes. He used those

Then, of course, you can measure the length of a folium in the cerebellum. It is a little easier place to count cells than somewhere else in the nervous system.

In some parts of the nervous system, then, not among the Purkinje cells, we find a peculiar relationship between the smaller glial cells and the big nerve cells when those nerve cells are degenerating This has been known for a long time As a matter of fact, in Metchnikoff's book, he shows some figures of phagocytic cells, as he called them, consuming the higher cells of the body, in old individuals

In poliomyelitis, we see an invasion of the phagocytic blood cells into the nervous tissue, and the nerve cell seems to melt down before



FIGURE 2 Comparison of the cells of the semilunar (Gasserian) ganglion in young and old mice (A) Cells of a 40-day male mouse (B) Cells of a 693 day female mouse The decrease in amount of Nissl material, loss of clarity of the nuclear sap, and vacuolization of the cytoplasm are seen Reprinted, by permission of the Cambridge University Press, Publisher, from Andrew, W Cytological changes in senility in trigeminal ganglion, spinal cord and brain of mouse *J Anat* 75, 406 (941)

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In poliomyelitis, we see an invasion of the phagocytic blood cells into the nervous tissue, and the nerve cell seems to melt down before

This process can also be greatly heightened in inanition, at least in the mouse. In starved animals, you see this process going on

But in the cerebral cortex, and in the ganglion, and in the spinal cord, the general fact that there are changes in the nerve cells with increasing age could be seen; and these changes did include a loss of Nissl material, which, of course, is a relative thing, changes in the nuclei and nucleoli, and increasing numbers of degenerate-appearing cells, and cells being taken care of, apparently, by these smaller glial cells.

Lansing: How do the nucleoli change?

Andrew. In the majority of the nerve cells of young animals, the nucleolus looks very heavily stained. I really think that's because of the greater amount of chromatin on the periphery of the nucleolus, because the central part is still a light-staining structure.

Gey: It does present a vesicular appearance?

Andrew: Yes, if you get to the central portion itself But there is so much more chromatin around it in the younger animals, that it is more conspicuous It seemed more conspicuous to us.

While there are, then, these general changes in nerve cells, there are definite differences in different parts of the nervous system, certain things which are characteristic of aging in particular groups of nerve cells. In the cells of Purkinje, for example, amitotic division of the nucleus is a rather characteristic thing in rodents The massing of pigment in cells is a rather characteristic thing for the ventral horn cells in man and in the lower animals. Sometimes, almost 100 per cent of old individuals will show a large amount of pigment.

Engle. Dr Andrew, do you know anything about the nature of that pigment, or its metabolic origin?

Andrew It has been called senile pigment, and I believe it is referred to as a lipofuchsin pigment I think that Dr Lansing probably knows twelve times as much about it as I do

Would you care to say a word, Dr Lansing?

Lansing. They are called hemofuchsin, and they are highly insoluble I believe that is all we do have of a factual nature about these pigments

Andrew Is that the same type of pigment, would you say, as is found in old cardiac muscle, or old liver cells, or do you think this material in the nerve cells is different?

Lansing These materials appear histologically to be similar, although the evidence for their chemical similarity is lacking There is no reason to doubt that they are similar materials.

Wislocki They accumulate particularly in cells which are rich in lipids You find these pigments in the inner zone of the adrenal cortex and in various ovarian cells.

the attack of the phagocytic cells. Perhaps the nerve cell is already dead, and it is simply being removed. It was interesting to us to find more of this type of picture in the senile nervous system than is ever seen in the younger person.

Figure 3 shows a cell from the cerebral cortex of a 75-year-old individual, and shows a rather high degree of what is called satellitosis A number of these satellites surround this nerve cell, which shows the rather degenerate characteristics of the nucleolus and the nucleus. We even find tiny remnants of nerve cells We could follow by serial sections to show that they generally still include Nissl material.

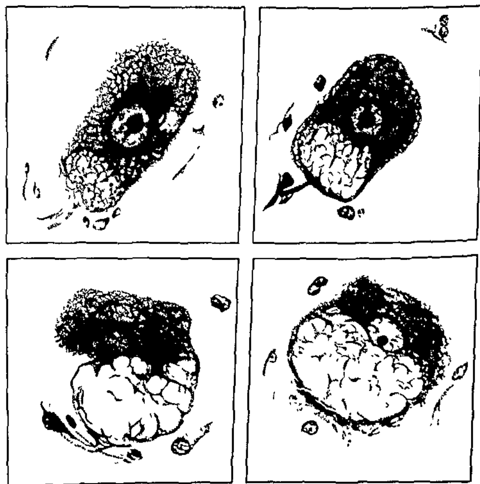


FIGURE 3. Stages in the process of fatty degeneration in the semilunar ganglion in old age. All figures from human female, 75 years old Redrawn from Truex, R, C Morphological alterations in the gasserian ganglion cells and their association with senescence in man *Am J Path* **16,** 255 *(1940)*

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Wislocki: They accumulate particularly in cells which are rich in lipids You find these pigments in the inner zone of the adrenal cortex and in various ovarian cells.

Lansing: And yet, they are most prominent in cardiac muscle fibers

Wislocki: In cardiac muscle fibers as well as in the central nervous system.

Lansing: Indeed, I think one might find them in any tissue that is studied.

Wislocki: But especially in tissues rich in lipids

Andrew: I might interject that we almost always have thought of the pigment as chiefly in the cardiac type of muscle. At least, I did, until I read some articles on the striated skeletal muscle which showed an increase in pigment, in work done by Bucciante and Luria (6), which showed that there it is about as conspicuous a phenomenon as in the cardiac muscle.

Fremont-Smith: I am struck by the statement that the pigments are highly insoluble. I cannot help wondering whether it does not suggest that the reason they are there is because they are so insoluble the body cannot get rid of them They might conceivably be secretions of some sort, which are metabolic. This pigment is a residual which remains in the cell because it is chemically insoluble to the solvents available in the cell.

Lansing: Of course, that is really the heart of a rather old theory of aging: that if an insoluble substance forms in the cytoplasm, or indeed, in the nucleus of a cell, it cannot get out. Such materials can only accumulate with time, and theoretically, then, obstruct the vital processes It is a form of the intoxication theory of senescence.

Carlson: Dr Andrew, may I ask you: do these changes which you have described here in the Purkinje cells, and other cells in the nervous system, apply to the nerve cells in the short-lived animals, such as the mouse or the rat or the honeybee?

Andrew: I think that is one of the fascinating things about changes in the nervous system, that here we have cells that are born, we might say, with the organism They cease their reproductive activities close to the time of birth, or, as shown in the mouse, slightly afterwards, and they live as long as the organism, if they do not undergo this degenerative change. Nevertheless, the changes seen in man, who may live 90 or 100 years, and in the rat, which lives, say, three years, and in the mouse, which lives two years, are quite similar. They are the same general type of change, so that these nerve cells have those respective spans of life.

If we pause to ask the question, whether the life span of the nerve cell is conditioned by the life span, or life duration of the organism is determined by the life span of the nerve cell, we have two horns of a dilemma. I do not think that we can answer that question, but the answer to the question of whether or not the changes are similar in animals of varying life spans is certainly "yes."

Wislocki: That is a very interesting question. At a previous meeting the question was brought up by McCay and Saxton as to what extent rats or hamsters really reach old age, because of the nature of the intercurrent diseases to which they are susceptible and which kill them prematurely There was, if I remember correctly, some question as to whether that type of animal ever reaches extreme old age in the sense that man does Fifty years ago, before the control of infectious diseases, the average human life expectancy was somewhere in the fifties, and a century before that, in the forties, whereas now, it is in the late sixties

Andrew: Of course, there is still a fairly relevant question as to whether man reaches his natural span of life He certainly leads a fairly sedentary existence, also. I doubt whether we move more than rats do

Wislocki: We might take the female, and let the reproductive period be the point The menopause in the human is around, say, 45-55. What part of the female rat's life follows the cessation of the ovarian cycles? What fraction of the total 600 days that the female of that species lives, follows the cessation of the ovarian activity?

Engle Well, of course, the human female varies so much from all the lower animals, because the human female enters the menopause and ceases menstruation because she has no more eggs, and the ovary cannot produce hormones unless eggs are present There is no other animal that I know of that loses all of her eggs as she comes into the last third of the span Macaque monkeys which I have studied, that have been grisly old *grandmas*, still have cortical ova, and go ahead with sporadic menstrual cycles at any age That is the one respect in which the human female differs from all others

Andrew As to this matter of not being quite sure whether or not the oldest laboratory animals that we have are senile animals, I feel pretty confident, myself, that they should be described as senile, and that they are close to the end of a natural life span, or at least, relative to what we know in man, but it points up again the very sore lack of a supply of old animals kept under favorable conditions, which is such a thorn in our side in trying to work in gerontology. It certainly would not be too hard to keep animals, and under some conditions in which they would be more closely simulating a natural type of life However, instead of that, we actually do not have animals that have even been kept in cages throughout their lives. They are just not available

Hoskins Is it implicit in your presentation, up to this time, Dr. Andrew, that these cytologic changes can be correlated with functional changes? Are you implying that?

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Andrew: I think that would be implied, yes.

Hoskins: In that case, I should like for a moment to hear Dr. Fremont-Smith's reflections on such things as Alzheimer's disease in man, which is marked by degenerative changes, and the relation of the structural changes in the brain to function.

Fremont-Smith: No, I am not the person to reflect on that, but I am sure there are others here who can. Wouldn't you do that yourself? You have given a great deal of thought to that.

Hoskins: Well, as a matter of fact, in the human situation, the correlations are not very satisfactory. One can find—I mention Alzheimer's disease as an example—people whose brains at autopsy seem to be a pretty degenerate mess and who function pretty well, and other people who function very badly without showing very much of this type of degeneration. I use this point to bring out how far we are justified in reading into what Dr. Andrew is saying about implications of functional disability.

Fremont-Smith: Let me make one comment at this point, because I think this problem is too complex to try to make that kind of correlation in any specific way. Just take general paresis, about which we know a great deal. Here is an individual with a very sick brain. The natural tendency has been to correlate the function of the very sick individual and his mentality with his obviously distorted and sick, dying, dead, brain cells. He has cortical atrophy, and he has atrophy of cerebral function. Then we come along with malaria and penicillin —of course, previously we did have, but neglected, the spontaneous remissions—and all of a sudden, this man becomes practically normal again with respect to behavior. He is functioning pretty well, and all his gross bizarre behavior distortions disappear, and yet you know that none of the cortical atrophy has disappeared. Therefore, it is perfectly obvious that the functional correlation had to be made with the living cells, and that the pathology which you could see, which was quite gross, was fundamentally unrelated to the change in function.

Therefore, I think if we bring that question up, we are going to be lost, because the brain is a whole series of organs, and we cannot separate our functions and relate them to the organ of the brain which we are talking about.

Carlson: May I add a comment which will strengthen your comments, Dr. Fremont-Smith? In certain types of so-called psychic mental disturbances, we are actually improving a certain percentage of the cases by cutting of the frontal lobe.

Fremont-Smith: Exactly. It points it up very nicely.

Hoskins: It should also be pointed out that one of the most severe [illegible] at the functional level, that seen in schizophrenia, has not

been shown to have any kind of correlation at all with brain cytology.

Shock. Would not the use of tissue cultures offer a technique for circumventing some of the problems raised because of inter-relationships between different organs and tissues as they exist in the intact animal? Certainly the conditions are more readily controlled in a tissue culture In the past, the major interest in tissue cultures has been in the structural characteristics of the cells. With the many techniques now available, would it not be possible to study some of the physiological characteristics of cells grown in tissue culture? Perhaps this



FIGURE 3 Vesiculated nucleoli in living cells of a strain of human epidermoid carcinoma Explanted 8/11/51 Mag 2900 x

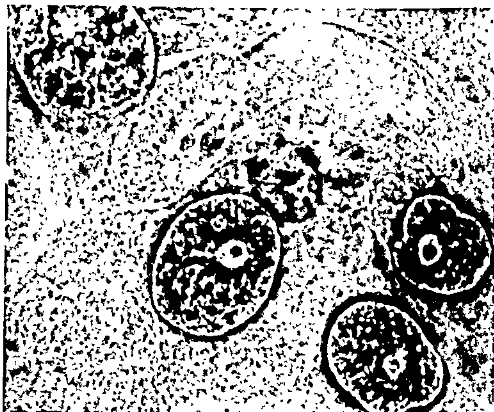


FIGURE 5 Vesiculated nuceoli in fixed cells of a strain of human epidermoid carcinoma Explanted 8/11 51 Mag 2900 x

approach would be of value in determining correlations between structure and function.

Gey: Dr. Andrew brought up a statement or two that has made me mull over them in relationship to other things He mentioned polio virus, and I hope that we shall mention other viruses as we go along in this conference

Variation in functional competence in cells, without *interference* with processes due to disease, does not seem to fit into my thinking Man is constantly exposed to a number of ubiquitous harmful agents, and microorganisms are among them.

Now, what do some viruses do to cells? In the first place, Dr. Andrew mentioned the nucleolus. In radiation injury, one sees vesiculated nucleoli. I called this to the attention of Dr Warren Lewis in the mid 1930's, and he has since reported very nice examples of vesiculated nucleoli in cultured cells (7). In fowl pox also, one sees vesiculated nucleoli (8). The virus is able to get into the nucleus rather easily.

In strain cultures of carcinoma of the cervix, we see greatly vesiculated nucleoli (Figures 4 and 5).

In exposing strains of cells to various viruses, we find that many cells are killed (9, 10, 11). These cells often calcify (11), and they remain in our cultures for long periods This is the kind of material that was brought up a moment ago as a rather difficult thing to get rid of, and one wonders whether, in many onslaughts that man experiences in various infections and in trying to get rid of them, some of these deposits don't actually stay there for a very long time.

I can also give you evidence that certain types of injury, such as



FIGURE 6 Appearance of living roller tube culture of Rat Sarcoma 319, 48 hours after last transfer and 126 days following exposure to 1000 r of X-rays Mag 255 x



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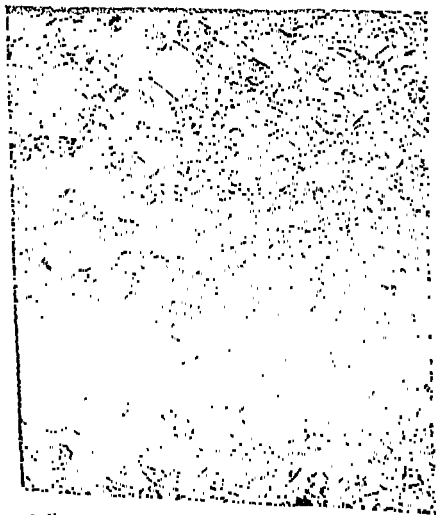
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because at the time the photographs were taken, they were in an ordinary test tube

In Figure 6 we see a strain of cells which, even in an ordinary test tube, shows a number of branching processes It is a strain of Walker Rat Sarcoma 319 (12). One might call this a cell derived from a reticular fibroblast. This is a strain that Mrs. Gey has to date cultivated for well over 20 years. If one exposes these cells to 1000 r (Figure 6) and 4000 r of X-rays (Figure 7) and examines the preparation about 120



[illegible] Roller tube culture of Walker Rat Sarcoma 319, 134 days following 1000 r of X rays Cells apparently recovered Mag 255 x

radiation injury, are extremely difficult to get rid of. If one gives sublethal doses of radiation to strains of cells and carries these cells on for months afterwards, he gets only slow recovery. In fact, not until some six months or so after he has given the radiation.

Carlson: When you say the effect of radiation, you mean the chemical residual, rather than the functional effect?

Gey: Yes, the chemical residual or, let us say, the cytopathological lesions that remain. I am not proud of these as cytological specimens,

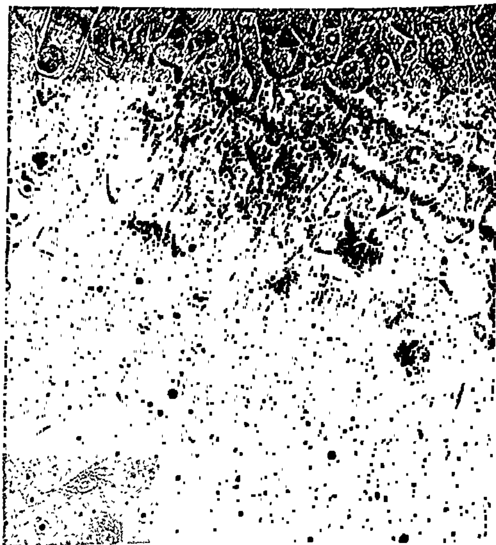


FIGURE 7 Similar culture 48 hours following transfer and 120 days after 4000 r of X-rays *Note greatly swollen vacuolated cells* Culture exposed to 1000 r shows several dividing cells Mag. 255 x.

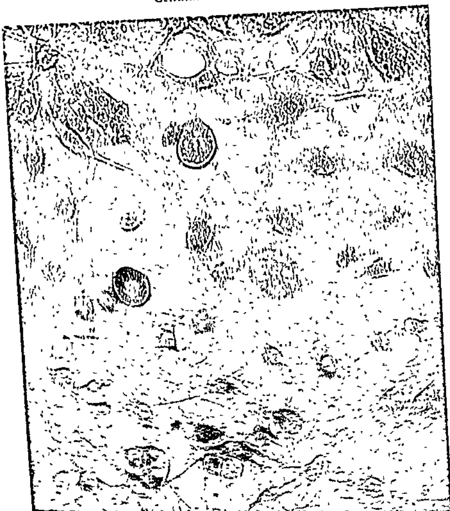


FIGURE 10 Similar culture 134 days following 4000 r of X-rays This shows a dividing cell and greatly swollen cells in this living culture Mag 255 x

1000 r, 2000 r, and 4000 r (and mind you, the maximal dosage, or lethal dosage, is somewhere around 6000 r), these cells, from 120 to 134 days following radiation, are still greatly swollen in the 2000 r and 4000 r group. I must remind you that for this strain, a dose of 6000 r approximates the 100 per cent lethal dose

Fremont-Smith Are these the same cells, or are they divided and duplicated?

Gey. There is some division going on There is a dividing cell in Figure 10 There is a very slow division rate going on in these irra-

days later, one finds that the cells are still swollen from radiation injury in the 4000 r group.* This persistence of a damaged state is still present even though we have transferred them from tube to tube serially. In the case of several of these specimens (Figures 8, 9, 10, 11) given

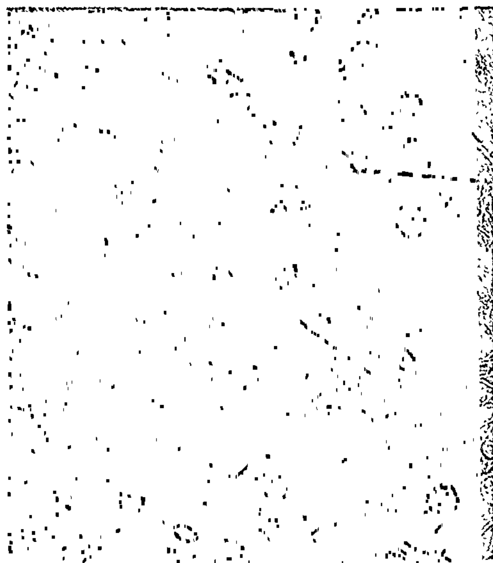


FIGURE 9. Similar culture 133 days following 2000 r of X rays. Cells slightly swollen. Mag. 255 x.

*This study of the effects of radiation on strains of cultured cells was supported by the International Cancer Research Foundation of Philadelphia and only a few of these results were given in their annual report of 1935. The work was also supported by the Carnegie Institution, Department of Terrestrial Magnetism, and some of it was done in collaboration with Dr. Merle Tuve and Dr. Larry Hafstad before World War II. We hope to be able to report our complete findings at some future time.

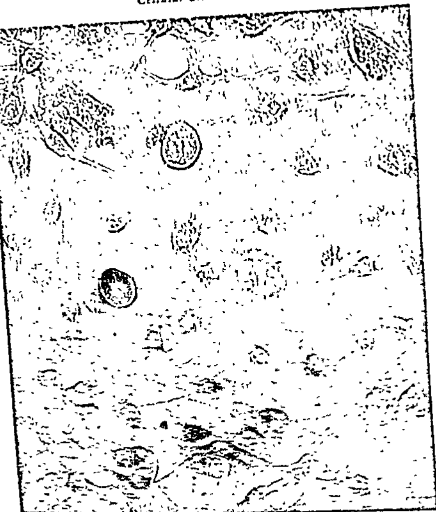


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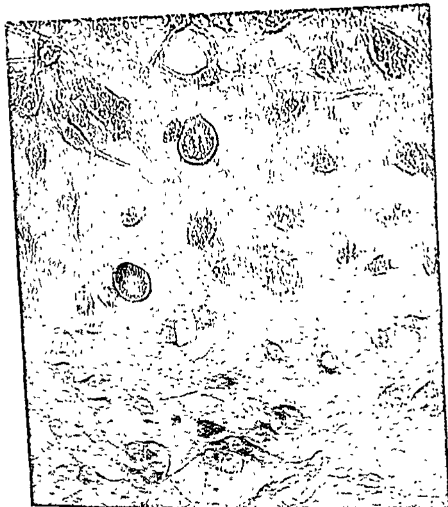


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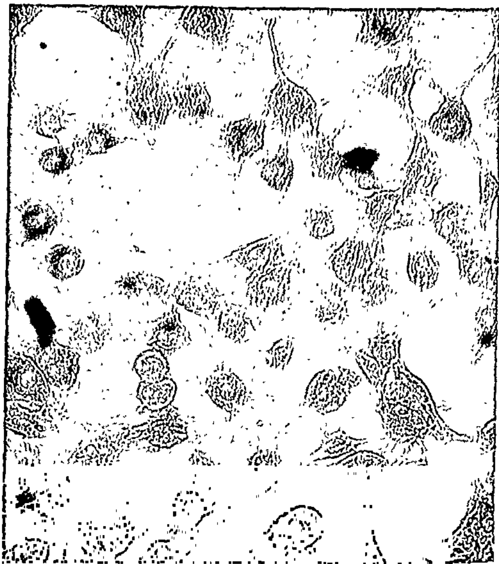


FIGURE 11. Similar culture 134 days following 4000 r of X-rays Note the greatly swollen cells in this living culture Mag 255 x.

diated continuous roller tube cultures even though serial renewal of medium had been practiced.

This swollen condition and slow growth was observed to be present for about half a year before the entire preparation got back to "normal" and restored to us the original strain as though nothing had ever happened. However, it took a long time even under the conditions of stimulation by renewal of medium to get these cells to come back

Now, under the conditions of dormancy that one finds in an intact host, how long a restoration period would be required for this tissue

to get back to where it was originally? Or of a normal tissue? This is the only point that I wished to emphasize.

Hoskins: Is the proliferative factor coming in?

Gey: There is very little proliferation (cell division) in these cultures. During the protracted injury period, growth is greatly retarded, as compared to the controls.

Figures 6 and 7 show a low-dose experimental tube 126 days following 1000 r and a high-dose tube 120 days following 4000 r of X-rays The low dose of 1000 r compared with the 4000 r irradiated culture shows, long after the radiation, greatly swollen cells in the high dose group. These cells are full of droplets, evidently lipoprotein vacuoles which may be swollen mitochondria They are not easily gotten rid of. They remain in these cells, no matter how good is the medium used for renewals The medium used was certainly able to keep cells in the control cultures growing progressively. We had many dividing cells in the controls and low-level irradiated cultures. The control grows in the order of tenfold in 10 days, a rather rapidly growing rat sarcoma. I am sure that one could explore with this kind of material and method the possibilities in terms of cumulative cell damage, regardless of what the injurious agent might be

Lansing: Is it your point, then, that aging involves an accumulation of injuries that are products of external forces?

Gey: I would say there are two things This is one kind of thing This can be accumulative In the case of viruses, one gets also into the carrier situation where persistence of virus is a factor.

Lansing: That still is an external agent—the virus

Gey: That's right.

Lansing: Are you postulating, then, that external sources of damages—

Gey: What I am saying is that this picture is a most intimate relationship to the cellular pathology of aging, as I see it now These forces are the things that get right into the cell There isn't a part of a cell that I could think of that is not interfered with by a virus. The damage seen is sometimes a complete dissolution, with the organism being able to get rid of all the residual products In many cases, calcified masses of material develop, which I feel pretty certain are rather difficult for the cell to get rid of

The other situation is this carrier state, which one ma[illegible] l in a great [illegible]

[illegible] saying that the accumulation of damages with age that occurs over and over again does affect the competence of a tissue to regain its original composition, as of some period in the past



FIGURE 11 Similar culture 134 days following 4000 r of X-rays. Note the greatly swollen cells in this living culture Mag 255 x

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not paid too much attention to differences between these organs. They describe the organ of a young, or of a senile individual, and describe the pathological changes without so much reference to possible effects of aging

We have felt that in studying rather large numbers of animals, [illegible] whether the organs were from young, middle-aged, or senile [illegible] and that there were rather definite criteria, sometimes rather subtle, and other times, very marked. We have tried, in some cases, to extend this work to human tissues, usually with some result that we thought was more or less fruitful

Gross. Dr Andrew, in relation to these subtle changes you mention, don't you feel that perhaps they are related in some way to this discrepancy between the marked pathological damage and the minimal functional disability one occasionally sees as well as the converse—the point that Dr Hoskins and Dr Fremont-Smith discussed? Isn't it possible that the methods used at the present time are just inadequate to show up the damage that counts The subtle changes in the remaining living cells may provide the answers

Andrew. Well, I very much believe in the furthering of our technical methods wherever possible and the application of them to problems of this type I think it should be occasionally pointed out that there are other deficiencies besides those of technique, and one is simply the matter of deficiency in observation of large amounts of properly controlled material I do feel that there are still a great many things to be found by using the so-called classical methods of histology and cytology, which still, as a matter of fact, are not at all old They have existed for a relatively few number of years

Fremont-Smith Just in that connection, isn't our richest opportunity in the integration of the newer techniques with the so-called older techniques, rather than saying that one or the other fails? For instance, this came out in discussions at our Conference on Connective Tissues The provocateur started off the first session with a definition of ground substance, and practically the rest of the morning was spent on that, because there was absolutely no agreement at all The electronmicroscopist had his definition, the cytologist had his, and the definition was based on entirely different characteristics, entirely different methods.

Gross. I think the criterion for the best definition of a biological structure should be its utility, how useful is it in giving a functional answer, an operational answer? For example, take the question of pigment which you brought up Apparently, people have felt for some time that this insoluble pigment is an important feature of the aging

Lansing: Are you implying that in the absence of these external factors, the tissues may live indefinitely?

Gey: I should say that might be the correct answer.

Andrew: I think this emphasizes how our lack of knowledge in other fields contributes to our lack of knowledge in aging, because certainly the whole field of possible relations of viruses to cells, during what we consider their natural life span, remains not thoroughly explored.

I wanted to make one other comment about the matter of the accumulation of pigments. It is of some interest that among various groups of invertebrates, accumulation of pigments is bound up with the excretory process We may learn more about that later during this conference, but I recall that one type of invertebrate produces certain brown bodies that seem to be just about the only excretory product they have, and these are rounded balls of excretory material that are then passed out There are other examples of the relation of pigment to the excretory process in lower forms of life

I was stating that in the nervous system, we have these various changes, and it is an interesting fact that in different parts of the nervous system, there are differences in the type of change. I mentioned the tendency to division of the nucleus in the Purkinje cells, and then this accumulation of pigment in the ventral horn cells

Returning to Figure 3 (p 32), reproduced from some work by Ray Truex (13) one can see that in sensory ganglia, we have different stages of what is true fatty degeneration of single cells, where the nucleus is pushed to one side and fat is accumulating, and finally one sees a cell that is hard to recognize as such Truex has shown that there is a considerable amount of this degeneration in the sensory ganglia, but has not been able to find it in autonomic ganglia nor in other parts of the nervous system

The whole question of how marked the histological changes in different tissues are, is an interesting one and a difficult one on which to be very definite During the course of our interest in aging, we have studied the literature and carried out projects on diverse organs, such as the thyroid, liver, pancreas, and lymphoid organs, including the lymph nodes and the spleen, and more recently, the salivary glands I know that it is sometimes asserted that the differences which we see in looking at young and old tissue under the microscope are fairly subtle, and sometimes minimal One person who has, at times, made that point is Dr. Lansing, for whose opinions on any subject I have a very profound and abiding respect I think there is a good deal of truth in this contention, particularly in certain organs, such as the liver and kidney, in ordinary human material. In general, pathologists have

not paid too much attention to differences between these organs. They describe the organ of a young, or of a senile individual, and describe the pathological changes without so much reference to possible effects of aging

We have felt that in studying rather large numbers of animals, organs usually from about 50 to 100 animals of pedigreed strains, that we could come to the point where we felt that we could tell fairly well whether the organs were from young, middle-aged, or senile animals, and that there were rather definite criteria, sometimes rather subtle, and other times, very marked. We have tried, in some cases, to extend this work to human tissues, usually with some result that we thought was more or less fruitful

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tional histologic techniques, tetrazolium reveals interesting abnormalities of the enzyme components.

Gey: It has been reported that antibody levels have been influenced by diet The antibody levels could readily be affected by the extent of the lipemia that exists. In this way, the nutritional state could affect the immunological state of the host from time to time

Andrew: These are very interesting questions I think it is fascinating the way the thoughts develop, because Dr. Wislocki's statement in regard to the tissues which are composed of short-lived cells, the blood cells, was going to form a part of my summing up sentences. Now it does not matter too much whether I finish or not, because actually it has been summed up

However, it is true that there are a lot of tissues in the body (in blood, in the bone marrow, and, of course, in the skin, the epidermis, the intestinal lining, and the lining of other tracts) in which there must be, even in old age, pretty constant renewal of cells, and the question of whether those cells are qualitatively different from the actively regenerating cells of younger individuals is certainly a worthwhile one. Some work at Bowman Gray School of Medicine on blood cell counts tends to show that there is practically no difference in the white count and in the differential white count in old people from those of younger people, but there is no study of which I know on intimate qualitative characteristics of blood cells of different ages

Thuringer has shown that the epidermal cells in old skin, (at least the parts that he studied) show a higher incidence of mitotic division than they do in the young skin, so apparently there has been no letdown Of course, we do not know how long the mitotic process is taking in that older skin, but the number of mitotic divisions was definitely higher.

Short: Which species?

Andrew: Human It was skin removed at surgical operations.

Hoskins I wonder whether Dr Carlson would not care to discuss a question previously raised—how much living a starving organism actually accomplishes That has been a subject of study in his laboratory

Carlson: Well, I do not have anything final on that Our most definite result seems to be injury from excess eating, rather than the analysis of undernutrition Excessive eating shortens life and favors cancer and other tumors Now, why, in the rat, moderation in eating, which gives the longest life span, should decrease cancer and other tumors, I do not know My only guess is that excess eating in addition to the weight that you are carrying, probably causes a strain on every cell in the body, but we do not know definitely We see an example of it